

THE FUNCTIONAL ORGANIZATION OF THE NEURONES
OF THE CEREBELLAR CORTEX

Owing to the complex nature of the experimental procedures employed in these investigations, the experiments to be reported here were carried out in collaboration with Professor Sir John D. Eccles and with Dr. Eusebio Sakai. However, I was responsible for the initiation of the experiments on stellate and Golgi cells as well as for the many fine "micro-lesions" and the

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Eccles J.C., R. Llinás and K. Sasaki: Excitation of cerebellar Purkinje cells by the climbing fibres. *Nature (Lond.)* 203: 242-244, 1964.

Eccles J.C., R. Llinás and K. Sasaki: Golgi cells inhibitions in the cerebellar cortex. *Nature (Lond.)* 205: 1265-1266, 1964.

Eccles J.C., R. Llinás and K. Sasaki: The inhibitory synapse

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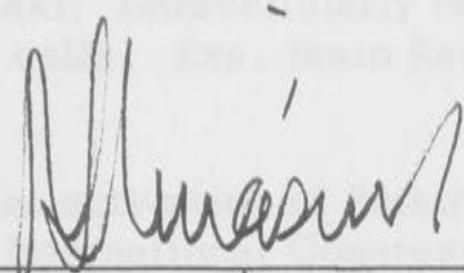
hibition as well as for the mossy fibre "axon reflex" and the

"climbing fibre reflex". The deafferented cerebellum preparation,

developed for the studies of the antidromic field potentials of

Purkinje cells and the field potentials generated by parallel fibre

stimulation, is entirely my own work.



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Eccles J.C., R. Llinás and K. Sasaki: The excitatory synaptic actions of climbing fibres on the Purkinje cells of the cerebellum. *J. Physiol* (In press) 1965.

Eccles J.C., R. Llinás, K. Sasaki and P. Voorhoeve: Interaction experiments on the responses evoked in Purkinje cells by climbing fibres. *J. Physiol* (In press) 1965.

Eccles J.C., R. Llinás and K. Sasaki: The actions of antidromic impulses on the cerebellar Purkinje cells. *J. Physiol* (In press) 1965.

Eccles J.C., R. Llinás and K. Sasaki: The inhibitory interneurons within the cerebellar cortex. *Exp. Brain Res.* 1; 1-16, 1965.

Eccles J.C., R. Llinás and K. Sasaki: Parallel fibre stimulations and the responses induced thereby in the Purkinje cells of the cerebellum. *Exp. Brain Res.* 1; 17-39, 1965.

Eccles J.C., R. Llinás and K. Sasaki: The mossy fibre - granule cell relay of the cerebellum and its inhibitory control by Golgi cells. *Exp. Brain Res.* 1; 82-101, 1965.

Eccles J.C., R. Llinás and K. Sasaki: Intracellularly recorded responses of the cerebellar Purkinje cells. *Exp. Brain Res.* (In press), 1965.

R. Llinás and K. Sasaki: Antidromic activation of Purkinje cells in the cat. Abstract 935 of the XXIII International Congress of Physiological Sciences. Tokyo, Japan, 1965.

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EPSP - excitatory post-synaptic potential
IPSP - inhibitory post-synaptic potential

Stimulating electrodes:

IV - deep radial nerve
IO - inferior olive
IF - intermediate-lateral region
LCX - local cortical
LOM - lateral occipital nucleus
TF - transverse

Recording electrodes:

ME - microelectrode
SE - surface recording electrode

Abbreviations

Anatomy:

BC - basket cell
CF - climbing fibre
GL - granular layer
Glo - glomerulus
GoC - Golgi cell
GrC - granule cell
MF - mossy fibre
MGR - mossy fibre-granule cell relay
PA - Purkinje axon
PAC - Purkinje axon collateral
PC - Purkinje cell
PF - parallel fibre
PL - Purkinje layer
SC - stellate cell

Electrophysiology:

EPSP - excitatory post-synaptic potential
IPSP - inhibitory post-synaptic potential

Stimulating electrodes:

DR - deep radial nerve
IO - inferior olive
JF - juxta-fastigial region
LOC - local cortical
LCN - lateral cuneate nucleus
TF - transfolial

Recording electrodes:

ME - microelectrode
SRE - surface recording electrode

I GENERAL INTRODUCTION

One of the most challenging problems in developing an understanding of the physiology of the nervous system is the one concerning the relations between structure and function at the neuronal level.

In studying the structure of the central nervous system one cannot but be fascinated by the geometrical organization of the neuronal elements of the cerebellar cortex. This organization is the more amazing as the regularity of the relations between the different elements is constant throughout the extent of the cortex.

From the electrophysiological point of view the cerebellum represents a unique opportunity to study functional organization of complex neuronal chains.

The history of the discovery of the histology of the cerebellar cortex saw its dawn in 1837, when J. E. Purkinje, the Czech professor of physiology at Breslau described the existence of what he called "Gangliösen Körperchen" in the cortex of the cerebellum. These cells named after their discoverer, Purkinje cells, are the central element of the histological organization of the cerebellar cortex. Purkinje described these voluminous bodies as forming a clear-cut layer, one element in thickness, close to the surface of the cerebellar crust.

In 1865 Deiters described the axonal prolongation of the Purkinje cells, their collaterals being described by C. Golgi in 1886, with the use of his silver impregnation stainings. Besides the axon collat-

erals of these cells and the plexus formed by their secondary and tertiary branches Golgi described also in 1886 the existence of the large stellate cells of the granule layer, which are known today as Golgi cells. In collaboration with Furasi (Golgi and Furasi, 1886) he discovered the existence of the basket cells, but as in the case of the large stars of the granule layer, failed to recognize the mode of termination of their axons.

However, it was the arduous work of S. Ramón y Cajal which gave the histology of the cerebellar cortex its status as the best known structure of the central nervous system. This anatomist described the mossy fibres and the granule cells, as well as their synapse (1888). In 1890, he discovered the mode of termination of the Golgi cell axon of which he writes in 1904, "...se concentran especialmente en los islotes cerebelosos, en donde entran en íntimo contacto con las arborizaciones dendríticas de los granos". In 1888, he demonstrated the presence of climbing fibres in birds and in 1890 in mammals, illustrating their intimate relation with the dendrites of the Purkinje cells. In the same 1888 paper on the molecular layer of the cerebellum of birds, Cajal gave the first description of the parallel fibres and their origin as bifurcation of the axons of the granule cells. He discussed also in this paper the relationship between the depth of a granule cell in the granular layer and the depth of the parallel fibres which the particular cell generates in the molecular layer, as well as the mode of termination of the parallel fibres in relation to the Purkinje

cells and to the interneurons of the cerebellar cortex.

It is of interest to mention that although it was C. Golgi who first described the basket cells, Cajal discovered the basket arrangement of their axon terminal around the bodies of the Purkinje cells, this being the first time an axon had been seen to enter in contact with another nervous element of the gray matter. On the basis of this single histological finding Cajal arrived at three conclusions (1888), a) "nervous currents" can propagate between two elements by means of their contacts and thus, jump from one corpuscle to another, b) Axonic prolongation can be the place of emission of "nervous currents" and the soma the place of reception. c) The initial part of the axon can also be a place of reception. Finally the stellate cells of the molecular layer were first described by Smirnov in 1887, who described several types of them. Based on his studies, Cajal proposed in his monograph "La textura del sistema nervioso del hombre y de los vertebrados" 1904, two main systems of "currents" into the cerebellar cortex. The climbing fibre-Purkinje cell pathway, a very restricted and private connection between these two elements, and the mossy fibre granule cell "omnicellular" pathway in which the currents coming from the mossy fibres would activate granules and through the parallel fibres Purkinje cells and all neurons having dendrites in the molecular layer. The basket, stellate and Golgi cells would then

serve as an "avalanche conduction system" to boost the input from this omnicellular pathway to the Purkinje cell. The histological picture, at this level, almost reached completion after the works of the early anatomists. In recent years the introduction of new histological techniques such as the Nauta stain as well as the use of electron microscopy for the study of the ultrastructure of nervous tissue, have allowed further developments in the understanding of the morphological organization of this cortex. The new findings on the subject will be discussed in the body of this thesis.

The study of the functional organization of the different neural elements of the cerebellar cortex can be said to have started with the pioneering experiments of Dow in 1949. This investigator demonstrated that local stimulation of the surface of the cerebellar cortex produced negative potentials which were conducted along the main axis of the folia of the cerebellum and which he related to the activation of parallel fibres. However, the modern analysis of the functional characteristics of the neural elements of the cerebellar cortex begins with the microelectrode studies by Granit and Phillips (1956 and 1957) on the electrical activity of single Purkinje cells of the cat cerebellum. These authors were the first to have clear extracellular and intracellular records from Purkinje cells, identified by their antidromic invasion from the white matter of the cerebellum. Similar results were also obtained by Matthews, Phillips and Rushworth (1957), on the activity of the Purkinje cells of the frog.

II METHODS

A) Surgical techniques

The experiments to be described in this thesis were performed in cats ranging in weight from 3 to 3.5 kilograms. They were anesthetized with 30 mgm/kilo of sodium pentobarbital, administered intraperitoneally.

The radial vein was then catheterized in order to continue the barbiturate anesthesia throughout the length of the experiments, as well as to administer other drugs. A tracheotomy was always performed and a tracheal canulae inserted into place to allow artificial ventilation of the animal.

The cat's head was then fixed on a stereotaxic frame, the pelvis being suspended by an iliac clamp. A single craniocaudal incision of the skin of the head was performed, it's borders being sewn to a stainless steel ring in order to create a pool for the Ringer's solution. The temporal and neck muscles were disinserted and the cranium scraped of periosteal tissue.

The cerebellar vermis was exposed by a craniotomy which extended from the bony tentorium to the lambdoidal ridge of the occipital bone. In most instances, the craniotomy was continued to the foramen magnum. This procedure exposed two or three folia rostral to the fissura prima and

a large proportion of the posterior lobe folia. Recordings were restricted to the culmen and lobus simplex. Laterally, the craniotomy extended the whole width of the vermal region. This ample exposure was needed in order to allow the selection of a recording site in the area of maximum response to the multiple inputs under study. The craniotomy was performed with bone nibblers under continuously running warm Ringer solution in order to prevent air embolism.

B) General experimental arrangements

Figs. 1 and 2 illustrate diagrammatically the general experimental arrangements. Monopolar surface recordings were obtained with a spring mounted silver ball electrode (SR) that was insulated with P.V.C. except for the very small area in contact with the cortex. The stimulating electrodes included a juxta-fastigial electrode (J.F.) consisting of three independent bipolar concentric electrodes. A number 30 hypodermic needle tubing was insulated with epoxy-resin up to 500 μ from the tip and an enamel insulated internal nichrome wire No. 48 (S.W.G.) protruded about 700 μ from the end of the tube. These three electrodes were cemented together so that their tips were 1.5 mm apart, allowing in this manner a very localized stimulus at each of those three points, in the cerebellar white matter. Single bipolar electrodes were placed in the lateral cuneate nucleus and in or close to the accessory olive nuclei. This latter electrode will henceforth be re-

ferred to as the inferior olive electrode (I.O.). A very short flat-ended concentric electrode (2 mm in length and outside diameter 0.8 mm) mounted on springy wire, was utilized to stimulate the surface of the cortex (LOC.), and of an adjacent folium, the transfolial stimulating electrode (T.F.). These surface electrodes allow a very localized region of stimulation. The superficial and deep radial nerves were stimulated with platinum electrodes under an oil pool.

Once the optimum responses were recorded with the surface electrode, the latter was removed to a nearby region of the same folium; and, after covering the exposed area of the cerebellum with an agar gel, a microelectrode for depth recording was lowered into the region of maximum response. Microelectrodes were filled with 4 mol. NaCl for the laminar field analysis ($2\text{ M}\Omega$ average D.C. resistance) and with 3 mol KCl or 2 mol K citrate for the intracellular recordings ($10\text{ M}\Omega$ average D.C. resistance). In order to minimize the movement of the cerebellar cortex, a bilateral pneumothorax was performed after the animal had been paralyzed with "Flaxedil" and artificially ventilated. A bridge circuit (Araki and Otani, 1955) with a $100\text{ M}\Omega$ resistor facing the grid of the cathode follower was used to pass current through the impaled cell. An R.C. coupled amplifier with a time constant of 3 to 10 seconds was used for the potential recording. The current measurement was done across a $180\text{ K}\Omega$ resistor using a differential D.C. amplifier of high input impedance. The mem-

brane potential was constantly monitored with a D.C. amplifier feeding a voltmeter.

In the initial stages of this work there were histological controls (haematoxylin-eosin) of the stimulating electrode placements in the inferior olive. Once the "typical" olivary response was established, however, only occasional checks were deemed necessary, because the cerebellar responses evoked by the olivary stimulation were so characteristic.

C) Surgical technique for cerebellar deafferentation

In order to deafferent the cerebellar cortex a bilateral pedunculotomy was performed and a minimum post-operative survival time of eight days allowed before experimentation. The surgery was done under heavy "nembutal" anaesthesia (45 mgms/kg) and the cerebellum was approached from its caudal end by removal of the squamous plate of the occipital bone; the craniotomy was extended to the lambdoidal ridge. In order to obtain an ample exposure of the floor of the fourth ventricle, the caudal region of the vermis of the posterior lobe, from the fissura praepyramidalis, to the velum medulare posterior, was removed by suction. The ventro-lateral part of both cerebellar hemispheres was then removed until a clear view of the inferior colliculi was obtained. Care was taken not to injure the medulla, the pons or the quadrigeminal plate. At this stage it is possible to section by means of a blunt edged spatula all three peduncles bilaterally under

direct vision, and thus to spare as much as possible the circulation to the cerebellum. Following the pedunculotomy the neck muscles, clavo-trapezius and occipito-scapularis of both sides, were approximated in the midline and joined with the caudal edge of the temporal muscles, which had been previously severed at their origins, so that a protecting muscle sheath was created over the craniotomy. The skin was closed using metal clips.

All the cats operated upon underwent the typical decerebellate syndrome (Dow and Moruzzi, 1958) with a "dynamic stage" lasting from two to three days during which extensor rigidity and opisthotonos was observed. Following this state the characteristic atonic period appeared. The animals showed intentional tremor with dysmetria and nystagmus. Nystagmus was present specially during psychical excitation, e.g. when presented with food. All through the post-operative period, lasting up to 23 days, the cats were completely ataxic and unable to stand. A week after the operation, however, they were all able to support their weight, and even to climb the wire screen on the sides of their cages, thus showing that their flexor responses were not as impaired as their antigravity mechanism. The cats were fed through an oesophageal canula during the first week, after which they began to eat unaided.

Following the electrophysiological studies the cerebellum and medulla were fixed in formalin. Both Marchi and Nauta stain techniques

THE CLIMBING FIBRE AFFERENT SYSTEMS OF THE CEREBELLAR CORTEX

were used to study the degeneration pattern produced by the pedunculotomy. These techniques demonstrated that for the most part the degenerating fibres were restricted to the white matter of the cerebellum, leaving the actual cerebellar cortex intact. Also small portions of cerebellar cortex were removed at the end of the experiment, before the death of the animals, fixed in 4% osmic acid and imbedded in "Vestopal" for electronmicroscopical (E.M.) observations which were kindly performed for us by Professor C.A. Fox and Dr. D.E. Hillman of the Department of Anatomy, Marquette University. The E.M. studies demonstrated degeneration of mossy fibres leaving the granule cells intact. A striking observation previously described by Hátori (1964); Fox (personal communication) and Szentágothai (personal communication) was the survival of the cerebellar glomeruli of the granule layer which then consisted only of the dendrites of the granule cells and the axonal terminals of Golgi cells. There appears to be an increase in the synapses made by these axons on the dendrites of granule cells. We revealed very large synaptic contacts between the

enlarged portions of climbing fibres and the small branches of the Purkinje cells. (Szentágothai, (1964a, 1964b) and Fox personal communication).

Schellert and Schellert (1954) made an extensive Golgi study of the climbing fibres in cat cerebellar cortex and reported more extensive synaptic relations than those previously described (see below). To some extent these findings have been confirmed by Fox (1962) and by

III A THE CLIMBING FIBRE AFFERENT SYSTEMS OF THE CEREBELLAR CORTEX

The climbing fibres of the cerebellar cortex were first described by Ramón y Cajal in his study of the molecular layer of the cerebellum of the pigeon, (1888) and were further investigated in mammals by him (Ramón y Cajal and Illera, 1907; Ramón y Cajal, 1911) and many other neurohistologists of his time. According to this anatomist the climbing fibres are neurotubules which traverse the granular layer of the cerebellar cortex and enter in contact with the dendrites of Purkinje cells. Upon reaching the Purkinje layer the climbing fibre branches out in order to twine around each of the large primary and secondary dendrites of the Purkinje cell (Fig. 1) without entering in contact with the small spiny branchlets. This very extensive and intimate contact which resembles the pattern of a climbing vine was assumed by Ramón y Cajal to form a powerful synaptic junction on to the Purkinje cell. The study of the ultrastructural details of this synapse has demonstrated these assumptions to be quite correct. Ultrastructure studies have revealed very large synaptic contacts between the enlarged portions of climbing fibres and the smooth branches of the Purkinje cells. (Szentágothai, (1964a, 1964b) and Fox; personal communication).

Scheibel and Scheibel (1954) made an extensive Golgi study of the climbing fibres in cat cerebellar cortex and reported more extensive synaptic relations than those previously described (see below). To some extent these findings have been confirmed by Fox (1962) and by

Szentágothai (1964a, 1964b). The study of the origin of the climbing fibres using the conventional degenerative methods has proved to be technically, extremely difficult. As a consequence, several different origins have been proposed for the climbing fibres. On the general grounds of the widespread distribution of the olivo-cerebellar fibres Dow (1942) suggested that the climbing fibres originated in the inferior olive. All other cerebellar inputs being via mossy fibres, similar conclusions had been reached by other authors in the past, Brouwer and Coenen (1919, 1921) and Winkler (1923). However, it was not until the study by Szentágothai and Rajkovits (1959), that a sound experimental basis was provided for the olivary origin of the climbing fibres.

The present investigation has utilized this discovery of Drs. Szentágothai and Rajkovits, it being assumed that climbing fibres projecting to the anterior lobe of the cerebellar vermis could be selectively excited by an electrode located near the accessory olives (Jansen and Brodal, 1954). Stimulation of the inferior olive has been found to evoke electrical responses widely dispersed over the cerebellum (Dow, 1939; Combs, 1956; Jansen, 1957). The dominant potential was a surface negative wave with a latency of 3 to 5 msec, but considerable variations were reported in the potentials led from different parts of the cerebellum. Certainly the later positive potentials can be due in part to the spread of stimulus to other structures adjacent to the in-

ferior olive.

a) Potential field evoked in the cerebellar cortex by electrical stimulation in the region of the inferior olive

A series of potentials evoked by a stimulus applied through an electrode in the region of the inferior olive and recorded by a micro-electrode in the contralateral vermal cortex at various depths below the surface are shown in Fig. 3,A,B,C. This field is composed at all depths of an initial negative wave with a fairly steep rise and a slower decline on which small irregular spike-like potentials were superimposed. This negative wave declined to a small slow positive wave, which was relatively larger at deeper levels.

In about half of the experiments on potential profiles this late positive wave was dominant at the deeper levels (cf. Fig. 3B,C,D) there being only a small initial negativity below 250_{μ} .

When applying electrical stimuli to structures like the inferior olive which are surrounded by such a wealth of neural pathways there is always the possibility that the observed responses are contaminated by a spread of the stimulus. The diversity of the potential profiles recorded could be argued to be produced by such spread. Fig. 3B-D illustrates an experiment to test for the existence of this possible distortion. Three strengths of inferior olive stimulation (3.0, 2.1 and 1.5 times threshold) were applied for each recording level of the

microelectrode which prodded the cerebellar cortex in depth. At the superficial levels the respective potentials differed in size, but not significantly in configuration and in time course with those recorded deeper, due to a large initial negative wave and a later positive wave, which became dominant with depths from 230_{μ} downwards. However, at 330_{μ} and 430_{μ} the strongest stimulus (B) evoked a positive wave which being larger, suggested that it could be produced in part by a spread of stimulus to structures adjacent to the inferior olive, such as the reticulo-cerebellar fibres from the lateral reticular nucleus. Otherwise the potential profiles of Figs. 3B-D, may be regarded as being produced by the activation of the olivo-cerebellar pathway.

The latency of the negative wave in the superficial levels of the cerebellar cortex is so brief (3.9 to 6.8 msec in 4 experiments) that it must be produced directly by the olivo-cerebellar pathway, which has been shown by Szentágothai and Rajkovits (1959) to end exclusively as climbing fibres. Hence the potential profiles of Fig. 3 can be regarded as being produced by the impulse of climbing fibres and by the synaptic and action potentials generated by them on the Purkinje cells.

The large extracellular negative potentials at levels from 30 to 200_{μ} in Fig. 3A-D exactly correspond to the potentials that would be expected for a depolarizing synaptic action of the climbing fibre impulses on the Purkinje cells, for this range includes the levels of

greatest synaptic density. The decline in negative potential and even its reversal to a positive wave below 200_{μ} in B-D are attributable to passive sources at the somatic and axonic level of the Purkinje cell with respect to the active sinks of the main dendrites. In Fig. 3, it can be seen that at more superficial levels there is, correspondingly, a larger latency of the initial negative wave, which is attributable to the conduction time of the climbing fibres. An approximate conduction velocity of 0.15 to 0.5 msec was calculated on this assumption for the fine terminal branches of these fibres (four experiments).

The diphasic negative-positive potential produced by inferior olive stimulation at very superficial levels (Fig. 3) and actually on the surface (Fig. 4E) is in good agreement with the potentials recorded with gross surface recording (usually a negative-positive wave) which are found following such stimulation (Dow, 1959; Jansen, 1957). There are also subsidiary synaptic excitatory actions of climbing fibres on basket cells and on Golgi cells, findings which are in accord with histological observations (Scheibel and Scheibel, 1954; Szentágothai, 1964); but these actions could make, due to their weakness, only a small contribution to the potential field, and would in any case produce a much deeper negativity (about 400_{μ}) than the one actually observed. There is, however, the further possibility that inhibitory synaptic actions of basket (Andersen, Eccles and Voorhoeve,

1964) and Golgi cells (Eccles, Llinás and Sasaki, 1964b) may contribute to the positive potential fields at the deeper levels in Fig. 3.

Since the climbing fibres are slender and rather sparsely distributed, it would be expected that the action currents of these fibres would give a negligible contribution to the observed potential field, particularly as their range of conduction velocities would result in a temporal dispersion over several milliseconds. Furthermore, they should theoretically produce a potential field having an initial positivity and later negativity, not the initial negativity actually observed.

It seems therefore justifiable to conclude that the potential profiles of Figs. 3A-D are generated by the synaptic excitatory action of climbing fibre impulses on the dendrites of Purkinje cells, and that they are due in part to excitatory postsynaptic potentials, and in part to the impulses generated thereby. This provisional conclusion was corroborated by such data as selective extracellular recording from individual Purkinje cells.

b) Extracellular recording of spike potentials

As illustrated in Fig. 4A, inferior olive stimulation can evoke a complex series of spike potentials at a depth of 250 μ in the cortex of the cerebellar vermis. By careful adjustment of stimulus strength, this whole complex can be demonstrated to be a unitary response superim-

posed on a low background potential (Fig. 4B). In most cases there is an initial large diphasic spike potential (positive-negative) followed by a succession of spike potentials of the same polarity but of various smaller sizes. For example, in Fig. 4B there were four superimposed traces, two below threshold and two above. The two latter gave four successive superimposed spikes.

Since the Purkinje cells axons represent the only efferent system of the cerebellar cortex, a stimulus applied to the juxta-fastigial region (cf. Fig. 1) would be expected to excite the axons of Purkinje cells and of no other cells in the cerebellar cortex; (Granit and Phillips, 1956). In Fig. 4C, there is an initial spike potential (up-going arrow) having a configuration closely resembling the initial spike evoked from the inferior olive. In view of its short latency it can be concluded that this spike was an antidromic invasion evoked in the same Purkinje cell. Each subsequent spike potential in Fig. 4 had the same configuration as the antidromic spike in C, the smaller size being attributable to a large depolarization of the Purkinje cell and to the relative refractoriness at that high frequency of response. The all-or-nothing property of the spike complex in Fig. 4B establishes that a single neural element coming from the inferior olive has a powerful excitatory influence on this Purkinje cell, the synaptic depolarization being so prolonged that it generated three or four impulses, and so large that the spike mechanism was greatly depressed. The

histological description of the climbing fibre synapse would agree with such a response.

Other examples of unitary responses of Purkinje cells are shown in Fig. 4D, (E is the simultaneous recording from the surface of the cerebellar cortex) where the initial spike was followed by a relatively negative wave with small superimposed spikes. In Fig. 4F, G, two spikes of another Purkinje cell were superimposed on a relatively large, slow positive wave. The whole assemblage in F and G represents an all-or-nothing response superimposed on a field potential which is well shown in F. In Fig. 4B, D, F, G, the unitary potential complex is superimposed upon a background potential generated by more distant Purkinje cells. When the recording electrode is located in the cortex superficially the unitary potentials are characterized by spikes superimposed on a large slow negative potential. On the other hand, when the electrode is relatively deep (300 μ in Fig. 4F-G), the unitary potential is formed by spikes superimposed on a slow positive potential.

c) Intracellular recording from Purkinje cells

The intracellularly recorded responses in Purkinje cells to an inferior olive stimulus are in good agreement with the extracellular responses. For example the latency of the first spike in Fig. 5B indicates antidromic invasion from a juxta-fastigial stimulation and so provides Purkinje cell identification; the response to an inferior olive stimulation (Fig. 5A), was an initial spike with a latency of 5.8 msec

followed by a large long lasting potential on which several small spikes were superimposed. The whole spike complex evoked by inferior olive stimulation usually had a duration of about 5 msec. Fig. 5D gives another example of the initial spike and later EPSP and spike complex evoked in a Purkinje cell which was identified by antidromic invasion in response to a juxta-fastigial stimulus (Fig. 5E). The later spike of the Purkinje cell in Fig. 5E will be shown below to be generated by activation of the mossy fibre-granule cell pathway, and thus of the system of parallel fibres which in turn have a multitude of excitatory synapses on the Purkinje cell dendrites.

In deteriorating Purkinje cells inferior olive stimulation may evoke in an all-or-nothing manner complex depolarizing potentials as in Fig. 6 which could suggest repetitive synaptic action. However, the sequence of Fig. 6 A-F shows that these complexities diminish and virtually disappear as the cell further deteriorates and thus it can be concluded that they arise not from delayed synaptic bombardments, but from local responses which disappear as the spike generating mechanism is progressively depressed by the decreasing membrane potential.

However, there are genuine cases of repetitive climbing fibre bombardment of Purkinje cells in response to a single stimulation of the inferior olive. In Fig. 7 A a just-threshold stimulation evoked either an EPSP with a triple summit having a latency of 7.3 msec and successive peaks at about 1.5 msec intervals, or a double-peak EPSP

having a latency of 9.0 msec. With further increase in the stimulus strength (B) the latency was identical with the shorter value in A, and with further strengthening there was further reduction in latency, to 6.0 msec in C and to 4.4 msec in D. At the same time it will be noted that there was virtually no change in the latency of the potential (lower traces) simultaneously recorded from the surface of the folium within 1 mm of the recording microelectrode. The initial negative peak of the surface potential had a latency of about 5.5. msec throughout, and its latency of onset was as brief as 2.6 msec in D. It seems that the weaker stimuli to the inferior olive were exciting presynaptic pathways to the cell of origin of the climbing fibre, and that only with the strongest stimuli (D) was this cell directly excited.

One explanation of these step-like variations in latency could be the existence of several sequential relays in the presynaptic pathway, there being presumably the jump of one synapse in the earlier record of Fig. 7 A. Alternatively the axons of inferior olivary cells may have collaterals that excite other olivary cells. Actually there is evidence in Figs. 10 and 11 for such positive feed-back. The discharge of some olivary cells could in this way excite others and these in turn still others; hence the latency of discharge along a particular climbing fibre may include several recurrent relays, and these can be by-passed in serial

manner as in Fig. 7 as the stimulation is progressively increased.¹⁾

It must further be postulated that the repetitive synaptic peaks are attributable to the repetitive discharge of the olivary axon which provides the climbing fibre to the Purkinje cell under observation in Fig. 7. Such a repetitive discharge at about 500/sec in response to a single presynaptic volley is very frequently observed by direct stimulation of the inferior olive (cf. Figs. 7 and 12).

d) The projection from the inferior olive to Purkinje cells

The pathway from the inferior olive to the cortex of the cerebellum decussates in the medulla and enters the contralateral restiform body (Jansen & Brodal, 1954) traversing the cerebellar white matter in such a way that the climbing fibres going to the vermis should pass close to the fastigial nucleus. This expectation is confirmed by the finding that juxta-fastigial (J.F.) stimulation frequently evokes responses in Purkinje cells which are identical with those identified as being produced by stimulation of climbing fibres (CF) by means of the electrode in the inferior olive. Similarly Granit and Phillips (1956) found that "fastigial" stimulation often evoked large and prolonged depolarizations of Purkinje cells, which they named "D" potentials and which are now identifiable as climbing fibre responses.

1) Dr. M. Ito has shown by intracellular recordings from inferior olive cells, that this is indeed the case (personal communication).

In the extracellular records of Fig. 8 A-C, graded J. F. stimulation evoked, when weak, the antidromic spike response of a Purkinje cell (A). In B. the stimulus was just straddling threshold for a complex response, consisting of an initial spike potential in the cell which was antidromically invaded and a later series of two or three small spikes (C), the whole complex resembling the one evoked a little later in that same cell by inferior olive stimulation (Fig. 4, D).

Fig. 8 D gives another example of juxta-fastigial stimulation evoking first an antidromic spike potential and then a later spike superimposed on a prolonged potential. Subsequently the micro-electrode impaled this same Purkinje cell, and the juxta-fastigial stimulation then evoked (Fig. 8 E, F) an initial antidromic spike potential and about 3.6 msec later a similar spike potential, followed by a large depolarization on which small spikes were superimposed. These responses closely resemble those illustrated in Fig. 5 A-D from two different experiments. In fact Fig. 5 B and C provide two other examples of the responses evoked by climbing fibre stimulation by a J.F. electrode, the J.F. stimulus in C being below threshold for the axon of the Purkinje cell. The illustrations of intracellular CF responses are seen to conform to a standard pattern, which has been regularly observed when the cells are in good condition.

e) The latency of the unitary responses

The unitary depolarizations attributable to climbing fibres are produced by juxta-fastigial stimulation with latencies ranging from 1.2 to 5.0 msec, there being only two examples out of almost 100 in which longer latencies (5.5 and 6.6 msec) were observed. This range of variations from 1.2 to 5.0 msec would be accounted for, at least in part, by variations in the location of the J.F. stimulating electrode, which would considerably affect the length of the climbing fibre to the Purkinje cell under observation. However, in most experiments the latency range for CF activation of adjacent Purkinje cells varied by a factor of more than two for a fixed position of the J.F. electrode, so presumably the range in conduction velocity of the excited climbing fibres is largely responsible for the latency range. As will be described below, juxta-fastigial stimulation can also set up delayed unitary responses which closely resemble the initial one and such a delayed response may have a lower threshold than the initial one. Probably such delayed responses account for the two exceptionally long latencies reported above.

When comparison was possible between the unitary responses evoked by inferior olive and juxta-fastigial stimulation, they were found to be virtually identical, as may be seen in Figs. 5 A-D and 8 E, F. The only differences of significance were in the respective latencies which almost invariably were briefer for the juxta-fastigial stimu-

ation, and in the repetitive character often observed for the response evoked from the inferior olive (Figs. 7 & 12). In Fig. 5 B and C the latencies were 4.9 and 5.0 msec, as against 6.1 msec for A.

The differential latency of the two modes of stimulation (inferior olive and J.F.) has been measured for 79 Purkinje cells in which the complex depolarizing response could be evoked by both methods of stimulation. In 59 the J.F. response was between 1.0 and 3.6 msec briefer, while in 12 the differential latency was between 4.5 and 7.6 msec; and in 8 it was less than 1.0 msec, approximating to zero in two. Since the actual conduction distance between the two sites of stimulation was about 20 mm (range approximately 17 to 23 mm in different experiments), conduction velocities of 4.7 to 23 msec can be calculated for the nerve fibres concerned in the latency differential of 1.0 to 3.6 msec.

Wide ranges of stimulation strength through either the inferior olive or J.F. electrodes almost invariably failed to disclose the convergence of two climbing fibres on to a single Purkinje cell. But two out of more than 100 cells exhibited a clear superposition of two unitary responses as in Fig. 9 A-D. The first and second are displayed in the threshold-straddling series of Fig. 9 A and C.

In the other example two quite different all-or-nothing EPSPs were evoked in a Purkinje cell by the inferior olive (E, F) and J.F. stimulations (I, J): With combined inferior olive and J.F. stimulations these two responses were summated at even the briefest stimulus intervals (G, H, K, L). Evidently the two modes of stimulation were exciting

different climbing fibres which innervated the same Purkinje cell.

f) Other modes of activation of climbing fibres

Fig. 10 shows intracellular recordings of the double unitary responses (arrows indicating both first and second responses) often evoked in a Purkinje cell by a J.F. stimulation, there being an interval of 6.0 to 6.5 msec between the two responses. Presumably the depressed size of the second unitary response is attributable to its superposition on the residual depolarization following the first response. The double character of the synaptically evoked response in Fig. 10 is seen very clearly when the deterioration prevented the generation of spike potentials by the neurone (D,E). Further examples of such delayed responses to J. F. stimulation are illustrated in Fig. 11.

The delayed response usually had the sequence of EPSPs, which are shown in the superimposed records to appear at regularly spaced intervals, just as in Fig. 7. In Fig. 11 A and C, stimulation through one J.F. electrode evoked an initial simple EPSP, and in addition the later response of 1, 2 or 3 EPSPs. In Fig. 11 B and D stimulation by the other J.F. electrode evoked only the later complex response. Apparently this electrode was relatively further from the climbing fibre supplying the impaled Purkinje cell, which consequently was not excited directly. The pathways responsible for these delayed actions of climbing fibres will be considered later.

In Fig. 12A, B a typical unitary climbing-fibre response of a Purkinje cell was evoked with a latency of 3.5 msec by stimulation of the inferior olive. The same unitary response with superimposed delayed components was evoked in C and D (note slower sweep speeds) by stimulation of the superficial radial and deep radial nerves of the ipsilateral fore-limb. The responses in C and D showed some latency variation, 12.2 and 13.2 msec in C and 11.5 and 12.9 msec in D. Fig. 12F gives another example of climbing fibre stimulation from a peripheral nerve, the superficial radial. The unitary character of the response is seen in the several superimposed traces, which are extracellularly recorded from a Purkinje cell, the minimal latency being 17 msec. The same unitary responses were evoked from the inferior olive in Fig. 12E with a latency of about 5 msec. Most of the latency differential between the peripheral nerve and the inferior olive responses would be attributable to conduction time in the rather long neural pathway, though presumably there are one or more synaptic relays.

When strong stimulation was applied by an electrode on the surface of that folium into which the microelectrode had been inserted, unitary responses of the typical climbing fibre character were sometimes observed to occur at a very short latency (Fig. 12G). Evidently, the strong stimulus had excited directly the climbing fibre supplying the penetrated Purkinje cell.

- g) Effect of polarization of the Purkinje cell on the excitatory postsynaptic potential generated by climbing fibre impulses.

Theoretically if the excitatory action of the climbing fibre synapse acts in the usual manner, that is, by creating temporarily a high ionic permeability (Eccles, 1964, pp.51-53), it should be possible not only to change the size of the EPSP by altering the membrane potential of the Purkinje cell, but even to reverse the EPSP, as has been done with a relatively few types of excitatory synapses (Coombs, Eccles and Fatt, 1965b; Burke and Ginsborg, 1956; Nishi and Koketsu, 1960). In Fig. 13 the EPSP produced in a Purkinje cell by inferior olive stimulation (series C) is shown in A to have the typical unitary character of a climbing fibre synaptic action, and even to have sometimes the delayed additional responses (B). In Fig. 13C hyperpolarization of the Purkinje cell by a current applied through the recording electrode with the aid of a bridge, is seen to increase the EPSP, while a depolarization reduces and inverts the EPSP so that the climbing fibre synapse evokes a large hyperpolarizing potential. Fig. 13D and E illustrate in another cell a better example of these large EPSP changes produced by changes in membrane potential. They were observed in two successive series of current application to the same Purkinje cell. E was recorded at lower amplification so that the potentials produced by the application of large currents could be applied. Under such conditions the two largest hyperpolarizing

currents restored the impulse-generating property of the deteriorated cell and so increased the EPSP that it was able to generate a spike discharge, which is seen as an all-or-nothing event in the second lowest trace.

It has not been possible to determine the membrane potentials produced by these applied currents; and, even if that had been possible, these measurements would obtain for the soma of the Purkinje cell and not for the region of the activated excitatory synapses on the dendrites. However, the applied currents were recorded, as may be seen in one trace of Fig. 13E for 3.6×10^{-8} A. The plotted points in Fig. 14 from the series partly illustrated in Fig. 13E are seen to lie along a straight line, there being an approximately linear relationship between the applied currents and the EPSPs on either side of the reversal point that occurred with a depolarizing current of 1.23×10^{-8} A. Such linear relationship has been observed in 3 of the 6 Purkinje cells in which this effect of current application on the EPSP has been successfully investigated.

B) Interaction between responses evoked in Purkinje cells by the climbing fibres and other Purkinje cell inputs.

a) Inhibitory action on the responses evoked in Purkinje cells by the climbing fibres.

Stimulation of the parallel fibres by a local surface electrode (Loc.) has been shown to produce a prolonged postsynaptic inhibition

of Purkinje cells of the folium under study, acting by the mediation both of basket cells (Andersen, Eccles and Voorhoeve, 1964) and of the superficial stellate cells (Eccles, Llinás and Sasaki, 1965b). Several procedures have been employed in investigating the influences of these postsynaptic inhibitions on the excitatory action of climbing fibre impulses on Purkinje cells.

The field potentials

As shown previously a single volley on the climbing fibre system (CF) produces a brief compound negative field potential with a maximum at a depth of 100 to 200 μ , while at depths below 200 μ there is a later and slower positive wave (cf. Fig. 15A). Furthermore, it was shown that this potential profile can be satisfactorily explained by the distribution of the excitatory action of the climbing fibre synapses on the Purkinje cell dendrites. This explanation assumes that, in general accord with the usual histological descriptions, the climbing fibre (CF) synapses on the Purkinje dendrites are most concentrated at a depth of 100 to 200 μ , with the consequence that both the EPSPs and the spike potentials generated thereby produce a maximum negative field potential at this depth.

In Fig. 15 the potential profile of A was not greatly altered in B when elicited during the postsynaptic inhibition of the Purkinje cells generally produced by a stimulus through the local surface electrode

(Loc.). It was surprising to find on the other hand that the inhibitory action increased the initial negative potential at all depths, and that there was a reduction of the later positive wave, particularly at levels deeper than 150_{μ} .

In Fig. 15B these changes of the field potential were tested at the fixed interval of 19 msec, which is probably a little later than the maximum inhibitory action on the Purkinje cells (Andersen, Eccles and Voorhoeve, 1964). In the series of Fig. C from another experiment the field potentials evoked by inferior olive stimulation were recorded at 150_{μ} depth and were tested at various intervals after a conditioning Loc. stimulation. The two control responses (CON) show the typical initial sharply rising negativity followed by a declining phase broken by small spike-like potentials: When conditioned by the preceding Loc. stimulation at intervals from 5.2 to 21 msec, the initial negative potential was increased in size (up to 160%) and had a much slower time course particularly in its declining phase. There was also a noticeable decrease in the superimposed small spike potentials. The maximal effect was at 13 msec interval, but it was still large at the longest test interval, 21 msec.

It has been an invariable finding that at test intervals beyond 10 msec the inhibitory action of Loc. stimulation does not decrease the height of the negative potential wave that CF impulses evoke from Purkinje cells; and usually, as in Fig. 15, there has been a considerable

increase. It is essential to examine the responses of individual Purkinje cells before attempting to give an account of these unexpected observations.

Extracellular recording from single Purkinje cells

In Fig. 16 the climbing fibre (CF) response evoked in an all-or-nothing manner in a single Purkinje cell (A, B) by inferior olive stimulation was depressed at a wide range of intervals after a conditioning Loc. stimulus. The initial spike response was unaffected, but the diminution in the subsequent partial spike complex was maximal at a test interval of 4.3 msec (D) and recovery was probably not completed even at the longest test interval of 33 msec (H). The briefest test interval (C) shows the frequent observation that at this short interval some facilitatory influence increases the subsequent partial spike complex so that it consists of three large spikes.

In Fig. 17 the climbing fibre response was evoked by juxta-fastigial (J.F.) stimulation, but there was the complication arising from the excitation of the axon of that Purkinje cell with the consequent production of an early antidromic spike potential (control in A) that preceded the CF response. At test intervals of 14 to 37 msec after the conditioning Loc. stimulus (E, F, G), the Purkinje cell was so deeply inhibited that the antidromic invasion was suppressed; yet the initial spike produced by the climbing fibre impulse survived undiminished.

However, as in Fig. 16, the subsequent partial spike complex was depressed, and had not fully recovered even at a test interval of 55 msec (H), by which time the antidromic invasion was restored.

Throughout Figs. 16 and 17 it will be observed that, in addition to this prolonged inhibitory action, the conditioning Loc. stimulation of the parallel fibres evoked itself a large initial spike and a variable subsequent partial spike complex. This is the expected response generated by the excitatory synaptic action which parallel fibre impulses exert directly on the dendrites of Purkinje cells (Andersen, Eccles and Voorhoeve, 1964).

c) Interactions recorded intracellularly from Purkinje cells

In the intracellularly recordings of Fig. 18 the Purkinje cell had already deteriorated, so that neither the conditioning Loc. stimulus nor the testing inferior olive stimulus evoked spike discharges. However, there were slow fluctuations of membrane potential with a periodicity of about 90 msec on which the responses of Fig. 18 were superimposed, as may be seen before the control response to the inferior olive stimulation in A. Furthermore, in G and H the periodicity of these fluctuations was reset at about 80 msec after the onset of the IPSP generated by the Loc. stimulation of the parallel fibres, (note that in A, D and F the inferior olive stimulation evoked a double climbing fibre discharge). In B-F the size of the EPSP produced by the climbing fibre (CF) impulse was increased when superimposed on the conditioning IPSP. In the same

manner in D and E there was also a considerable lengthening of the superimposed EPSP, which occurred to a lesser degree in C and F. On the other hand, when superimposed on the depolarizing phase of the background rhythm, the EPSP was diminished and shortened (G), there being again recovery in size and time course during the development of the subsequent hyperpolarizing phase (H). Other examples of this influence of membrane potential on the CF responses are illustrated in Fig. 19. For the present it is sufficient to point out that the potentiation of the CF-evoked EPSP during a conditioning IPSP could explain the increased negative field potentials that CF stimulation produced in Fig. 15B, C, under similar conditions, if some assumptions to be mentioned later are granted.

In Fig. 19 (CON) the control CF response to juxta-fastigial (J.F.) stimulation was superimposed on the IPSP produced by parallel fibre stimulation. It is evident that these CF responses were affected by the background IPSP in the same way as in Fig. 18C, D, E; which is well illustrated in the potentials recorded from this same cell at a slower sweep speed (G-L).

When the height of the CF-EPSP is measured from control background formed by the conditioning response, as in Fig. 19M it is immediately obvious that when the depolarization was superimposed on the initial phase of increasing IPSP, it was reduced in size (Fig. 19 B, C). On the other hand it was potentiated when it was evoked later, during the maximum hyperpolarization and the recovery therefrom.

These changes may be readily explained on the basis of the ionic conductance theories of excitatory and inhibitory postsynaptic potentials (cf. Eccles, 1964).

The decrease at testing intervals from 3 to 12 msec is doubtless due to the shunting effect of the high membrane conductance during the incrementing phase of the IPSP (upper trace of Fig. 19M). At longer intervals this raised conductance will decline progressively, and the size of the CF-evoked EPSP becomes more influenced by the increased membrane potential. The prolonged falling phase of the CF-EPSP under this condition will be considered later.

The IPSPs evoked by J.F. stimulation have the same action as those evoked from the Loc. stimulus, in conditioning the EPSPs produced by a CF impulse.

d) Double activation of climbing fibres

Since, in Purkinje cells with low membrane potential, climbing fibres evoke only simple EPSPs uncomplicated by spike potentials, this situation provides conditions which are particularly appropriate for testing the efficacy of a second CF impulse tested at various intervals after the first one. Invariably it has been found that there is a considerable depression of the second EPSP and that recovery from the depression takes some hundreds of milliseconds. For example in Fig. 20 with double J.F. stimulation, the second CF-evoked EPSP was 54% of the first at 6.5 msec interval (B), 75% at 42 msec (D) and was not

quite complete at the longest interval 410 msec (H). The full time course of the recovery process, except for the terminal phase, is exhibited in the curve of Fig. 20I. Similarly, in Fig. 21A-F with double stimulation of the inferior olive, the second CF-evoked EPSP was 70% of the first at 12 msec interval (A), and about 50% at the briefest interval for a second response, 1.4 msec (D).

When I.O. and J.F. stimulations evoke a CF response in a given Purkinje cell it is possible by the collision test diagramed in Fig. 21K to establish that the same CF fibre is responsible for both responses. For example, in Fig. 21 utilizing the J.F.-I.O. stimulus sequence the least interval for the second CF EPSP was 4.7 msec (F-J), while it was only 1.5 msec with the double I.O. stimulus (D). This increase in the least interval by 3.2 msec corresponds approximately to the latency differential for the respective J.F.- and I.O.-evoked EPSPs ($4.3 - 1.9 \text{ msec} = 2.4 \text{ msec}$). As shown in Fig. 21 K, the former value is a measure of the time for antidromic propagation from the J.F. to the I.O. sites of stimulation of that climbing fibre and the latter is the time for the orthodromic propagation on the same fibre from the I.O. to the J.F. site. This agreement would not be expected if an additional synapse were introduced on the I.O. pathway, because the I.O. stimulus would then act by exciting presynaptic fibres to inferior olive cells, but not these cells or their axons directly (Eccles, Llinás and Sasaki, 1965a).

e) Repetitive activation of climbing fibres.

As with double stimulation, the simplest conditions for investigation of repetitively activated CF synapses are provided by the intracellular recording of EPSPs from partially deteriorated Purkinje cells. In Fig. 22 A-E inferior olive stimulation at the indicated frequencies evoked typical unitary CF EPSPs, which were, throughout, uncomplicated by repetitive discharges from the inferior olive neurone. The second response of the repetitive series exhibited a depression of size matching those of Fig. 20, which is progressive for the first few impulses of the series. In the longer tetani of D and E the depression approached a steady level, which would be rather less than 40% of the control in D and about 32% in E. On cessation of these brief tetani there was no sign of the slowly declining depolarization which is characteristic of a lingering of transmitter action (Figs. 22, J,N,O; 23I).

The repetitive series of CF EPSPs in Fig. 22 F-J and K-O were evoked in another Purkinje cell by inferior olive and J.F. stimulation respectively. In the former series the first stimulus evoked a repetitive discharge of 4 to 5 CF impulses at about 500/sec, resembling those previously illustrated. In the second series the J.F. stimulation directly evoked a single CF discharge, which was followed by a typical repetitive "reflex discharge" from the inferior olive of 3 to 4 impulses also at about

500 /sec. In both series the subsequent stimulations of the repetitive series evoked only single CF responses which showed a depression of size which was more severe the higher the frequency. The relative depressions of the amplitude at the steady level were less than in the first series (A-E) - about 70 to 80% at 70/sec and 40 to 60% at 110/sec. In contrast with the first series there is clear evidence of a residual depolarizing action after cessation of the tetanus. This is particularly evident with the higher frequencies, 70 to 170/sec, where the depolarization persisted for as long as 25 msec after the summit of the last response.

Despite the depression of the EPSPs produced by repetitive stimulation, the CF synapses have an amazing ability to continue to produce repetitive spike discharges in Purkinje cells that are not injured by microelectrode impalement. For example, Fig. 23A shows the complex response produced by J.F. stimulation, there being an initial antidromic spike (a) and 3.2 msec later a complex of 3 spikes having the typical configuration of the CF-evoked response (cf). In B and C there was no diminution in the spike discharge (cf) evoked by a second CF impulse at 30 and 21 msec after the first, and, in the brief repetitive series of D at 70 msec, each J.F. stimulus produced a response not much depressed relative to the initial responses in B, C and D, these being the initial antidromic spike (a) and a later CF complex (cf) of three spikes. In the faster stimulation in E (108/sec) the CF complex with 3 spikes (cf) was

still well maintained for the 6 stimuli, but only the first stimulus evoked the initial antidromic spike (a). Presumably the propagation down the Purkinje cell axon of the last spike discharge of each CF response prevented by refractoriness the next antidromic spike.

With the still more severe tetanus of Fig. 23F (16 stimuli at 180/sec) there was a rapid failure of large spikes, and only irregular small spike potentials could be evoked during the remainder of the tetanus. However, immediately after the tetanic stimulation was arrested there was a remarkable after-discharge of large spike potentials commencing at a frequency of about 350/sec and declining to 180/sec just before failing. The increase in spike size with slowing of frequency may be entirely attributable to refractoriness. Comparable responses of Purkinje cells were observed by Granit and Phillips (1956) following tetanization by an electrode in the fastigial region, and likewise these may be attributable to the repetitive stimulation of climbing fibres.

Fig. 23 G, H shows similar responses evoked in another Purkinje cell of the same experiment described above, by quite brief tetani, 7 at 330/sec and 12 at 240/sec. In both cases there was the same deep depression of the Purkinje spikes during the tetanus and for about 10 msec thereafter, but subsequently a recovery of the spike size was evidenced. The spikes had a characteristic frequency pattern, which declined progressively from an initial high rate to as low as 100/sec (G) before failing, the frequency being higher after the 12 stimuli (H) than after 7 (G).

Finally in Fig. 23I intracellular recording from another Purkinje cell in this same experiment shows the large maintained depolarization during the tetanus (19 stimuli at 230/sec), and the slow decline for almost 100 msec thereafter, though there was no associated generation of spike discharge as in F, but merely a recovery of the spontaneous discharge frequency. This build-up of residual depolarization was also noted in the more severe tetani of Fig. 22 I, J, M, N, O, but was less well developed, presumably because this Purkinje cell was quite deteriorated.

It was assumed that in Fig. 23 repetitive activation of climbing fibres was responsible for all the subsequent after-discharge of the Purkinje cell due to the fact at lower frequencies the CF response was dominant (Fig. 23A-E). This characteristic type of response can also be produced by a brief tetanic stimulation of the inferior olive, and hence undoubtedly is due to climbing fibre activation.

C) DISCUSSION

There is a remarkable correlation between the present electrophysiological observations on the powerful all-or-nothing synaptic activation of Purkinje cells and the classical histological findings of the very extensive synaptic contact that each Purkinje cell receives from a single climbing fibre. There is no evidence in conflict with the conclusion that impulses in climbing fibre alone are responsible for these uniquely large

all-or-nothing responses of Purkinje cells that were originally observed by Granit and Phillips (1956). The physiological and histological evidence is further in agreement in showing that with rare exceptions (Fig.9) only one climbing fibre is distributed to each Purkinje cell. Again, the conclusion of Szentágothai and Rajkovits (1959) that the very extensive olivo-cerebellar tract is exclusively composed of climbing fibres is fully corroborated by the evidence that apparently pure climbing fibre responses of Purkinje cells can be regularly evoked by stimuli applied to the inferior olivary nucleus.

Several illustrations have been given (Figs. 7, 12A, B; 13B) of the frequent observation that stimulation of the inferior olive evokes a brief sequence of all-or-nothing responses at about 2 msec intervals. These responses are identical with responses evoked by repetitive activation of a climbing fibre hence, presumably, they signal the repetitive discharge of impulses along a climbing fibre from its cell of origin in the inferior olive. The direct or short latency responses evoked by stimulation of the climbing fibre by the juxta-fastigial electrode (Figs. 10D,E, 11) have never exhibited this repetitive character; yet, as shown in Figs. 10 D,E ; 11A-D, it was frequently observed with the reflex responses following juxta-fastigial stimulation. With such reflex responses there must be synaptic excitation of the inferior olivary cells; so it can be concluded that, when similar responses are evoked by stimulation of the inferior olive, the stimulus is exciting presynaptic fibres in that nucleus. Many monosynaptic relay stations in the central nervous system are now

recognized to evoke a brief efferent repetitive discharge by a single afferent volley, as indeed occurs with climbing fibre activation of Purkinje cells (Figs. 4, 5).

When juxta-fastigial stimulation leads to the "reflex" production of CF responses as in Figs. 10, 11, the latent period of these responses is always sufficiently long for a reflex pathway through the inferior olive. There are two simple pathways by which juxta-fastigial stimulation could lead to the reflex discharge of impulses from the inferior olive. In one the axon collaterals of climbing fibres would excite synaptically cells of the inferior olive. Fig. 11B,D shows that this positive feedback pathway via an axon collateral would have to go at least in part to cells other than the cell of origin of that axon collateral. The alternative pathway would be antidromic transmission down mossy fibres and thence by axon collaterals to inferior olive cells. It has been recognized histologically that many fibres give axon collaterals to the inferior olive as they pass by (Ramón y Cajal, 1909; Scheibel and Scheibel, 1954; Scheibel, Scheibel and Walberg Brodal, 1956) and many of these fibres could well be cerebellar mossy fibres.

However, this simple axon collateral pathway is not in itself capable of explaining the step-like shortening of latency that occurs in Fig. 7 with progressive increase in stimulus strength to the inferior olive, where actually four steps are illustrated. Only two can be ac-

counted for by the postulate that the weakest stimuli excite fibres that are presynaptic to the cells of origin of the climbing fibres, while the stronger directly excite the discharge of impulses along the climbing fibres. In order to explain the additional latency steps, it is necessary to postulate either serially arranged interneurons in the inferior olive or, more plausibly the pathway via positive feedback collaterals which can go through several relays of inferior olive cells before reaching the cell of origin of the climbing fibre under observation.

Direct investigation of the inferior olive has shown the latter case to be correct, Ito (personal communication). Further investigation is required before an explanation can be given of the very brief latency differential (less than 1.0 msec) that has sometimes been observed for the CF responses evoked by I.O. and J.F. stimulation respectively.

A remarkable feature of the Purkinje cell responses to climbing fibre activation has been the relatively long duration (about 5 msec) of the repetitive spike discharges evoked by a single impulse (Fig. 4 A,B; 5A-D; 8B,C,E,F). In contrast, in the deeply deteriorated Purkinje cell (Figs. 6F, 10D,E; 11A; C; 13A) the EPSP shows a relatively rapid and smooth decay to a low slowly decaying residuum. Under such extreme conditions it can be assumed that the membrane time constant is greatly shortened and that these brief EPSPs with a prolonged tail give an approximate indication of the time course of the synaptic trans-

Local mechanical injury by the juxtaposed microelectrode tends to suppress

mitter action. In the case of intracellular records from Purkinje cells in good condition, the depolarization produced by the synaptic excitation would have a longer duration; hence it seems possible to account for the rather long duration of the repetitive discharge of impulses observed under such conditions (Figs. 5, 6, 8) or with extracellular leads (Figs. 4, 12E,F).

In Figs. 4C, 8A-D, the spike potentials evoked by CF stimulation have a configuration (positive-negative diphasicity) that as usual closely resembles the antidromic spike potential. However, this resemblance merely shows that in both these cases the spike potential propagated into the region of the Purkinje cell under observation, which was the soma together with the large basal dendrites. It could be postulated that the spike evoked by the CF impulse was generated in the axon and propagated into the soma-dendritic zone exactly as with the antidromic impulse set up by J.F. stimulation. However, a more probable alternative is that the CF impulse generated the spike in the dendrites in the region of its maximum synaptic action at about 150 to 200 μ below the surface. The propagation of this spike down the dendrites to the soma region would give the diphasic positive-negative spike observed in this region (Figs. 4A-C; 8A-D; 12E). It must be recognized that the extracellular recording of giant spike potentials provides a misleading indication of the normal site of initiation of impulses. Local mechanical injury by the juxtaposed microelectrode tends to suppress

spike initiation at that site, and hence the invariable finding that such giant potentials begin always with an initial positivity (Freygang, 1958; Freygang and Frank, 1959; Terzuolo and Araki, 1961).

The field potential profiles (Fig. 3) indicate that a CF impulse generates the first impulse discharge in the Purkinje cell dendrites at the region of greatest density of climbing fibre synapses. Thereafter the situation is less clear. Intracellular recording invariably shows that subsequent spike potentials are greatly depressed (Figs. 5A-D; 8E-F), which is of course attributable to the severe membrane depolarization by the intense synaptic excitation. With extracellular recording this depression is also observed, but the configuration of the extracellular spikes in Figs. 4A,B; 12E,F; Fig. 23A-E certainly shows that many grow into propagated impulses. Presumably when the spike production of the soma and dendrites is depressed by the heavy depolarization, spike discharges can still be generated in the axon, just as is believed to occur for many types of nerve cell. This generation of repetitive axonal impulses would be expected to give the small spikes observed by intracellular recording. It is also possible that some of these spikes are generated in the more remote region of the dendrites.

The relatively slow time course of propagation of impulses in the slender climbing fibre branches is indicated by the increasing delay of onset of the negative field potential toward the surface (Fig. 3). How-

ever, this increase in conduction time would account for a range of less than 2 msec in the onsets of the EPSPs produced by the many synapses made by a single climbing fibre. Hence the relatively long duration of transmitter action with the prolonged low level tail can be attributed to the slow diffusion of the transmitter away from the sites of release and of action just as occurs for example in Renshaw cells (Eccles, 1964).

As mentioned above, large and prolonged unitary depolarizations were observed by Granit and Phillips (1956) to occur either spontaneously or after juxta-fastigial stimulation. These responses were called by them "inactivation responses" because of the suppression of spike generation during the strong depolarization. It could be possible to attribute all these responses to the very powerful synaptic excitatory actions of impulses in the climbing fibres. Furthermore, it is now clear from such records as those of Fig. 10A-E that with intracellular recording the typical inactivation response of Granit and Phillips is transformed into their typical D-potential as the impaled Purkinje cell deteriorates so that spike generation is suppressed. A similar interpretation can be offered for the D-potentials reported by Suda and Amano (1964). However, it must be kept in mind that ultimately the "inactivation responses" represent a physiological characteristic of the membrane of the Purkinje cell and that an understanding of this phenomena will only come about after more is learned about the characteristics of the sodium activation system in this membrane.

In their records of Purkinje cell potentials induced by juxta-fastigial and other types of stimulation Deura and Snider (1964) have recorded many complex potentials that may now be identified as typical climbing fibre responses. Juxta-fastigial stimulation often, as in Fig. 8, evoked a complex spike potential that now can be recognized as a unitary CF response. Furthermore, with juxta-fastigial stimulation, they reported delayed responses that correspond with the reflex responses in Figs. 10 and 11, having a latency of about 6 msec longer than the direct responses. The changes in potential wave form with deterioration of the impaled cell (Figs. 6, 10) were also illustrated by them. It is of particular interest that they found the same CF response to be evoked by cerebral as well as by J.F. stimulation. Evidently there is a fast pathway from the cerebral cortex (latency about 6 msec) for activation of climbing fibres.

According to the classical description of Ramón y Cajal (1911) a climbing fibre is distributed to only one Purkinje cell. On the contrary Scheibel and Scheibel (1953) report that a single climbing fibre may innervate adjacent Purkinje cells. Conceivably extracellular recording could provide additional evidence on this point. Innervation of adjacent Purkinje cells by a single climbing fibre would be established if the all-or-nothing climbing fibre response produced at exactly the same threshold was composed of spike potentials from two Purkinje cells. This has never been observed. However, this negative finding does not exclude multiple innervation because only occasionally

was the extracellular electrode located so that it could record from two Purkinje cells. In any case it must be emphasized that the present electrophysiological findings provide an excellent correlation with the usual histological picture, and reveal that the climbing fibre is a very powerful and specific excitatory synapse in the central nervous system.

With respect to the studies in the interaction between climbing fibres responses of Purkinje cell and other types of inputs it can be taken as established that the hyperpolarization due to the post-synaptic inhibitory potentials is produced by ionic currents flowing through the subsynaptic areas of high ionic conductance. It is postulated that through their inhibitory actions, basket and stellate cells produce on the Purkinje cells areas of high ionic conductance which would act as shunts for the currents generated by the CF synapses. It is thus possible to account for the depression of the CF-evoked EPSPs by the high ionic conductance that occurs during the rising phase of the IPSP in Fig. 19M. However, the hyperpolarization in itself increases the CF-evoked EPSP, there being thus two opposed actions of the inhibitory synapses. In Fig. 19M this incrementing action is seen to preponderate beyond 15 msec. The shunting inhibitory conductance would meanwhile have declined; nevertheless the membrane hyperpolarization would continue to increase before the later slow decline.

In Figs. 18 and 19, the CF-evoked EPSP exhibited a remarkable slowing in the falling phase when it occurred during the IPSP. The most probable explanation of this slowing is that the intense ionic currents produced by the activated CF synapses very effectively discharge the hyperpolarization of the IPSP, which later can be rebuilt only by the action of any inhibitory transmitter still lingering in the inhibitory synaptic areas. As would be expected from this explanation, the rebuilding in Figs. 18 and 19 was much more effective early in the IPSP than later, and there was little or no trace of it very late in the IPSP (Figs. 5K, L).

The excitatory power of a single climbing fibre synapse is demonstrated by its generation of an impulse discharge from a Purkinje cell even when it is subjected to the most intense inhibition from basket and stellate cells (Figs. 16, 17). With Purkinje cells under good conditions (extracellular recording) no inhibitory suppression of the first discharge to a CF impulse has been observed. But the later discharges can be very effectively inhibited.

Two explanations can be offered for the effect of inhibition in increasing and prolonging the negative field potentials (Fig. 15) produced by climbing fibre stimulation. There are firstly the increased and prolonged EPSPs that are observed during the hyperpolarization of the IPSP (Figs. 18, 19). Secondly, during the inhibition there is de-

pression of the later spike complex produced by climbing fibres (Figs. 16, 17). The more superficial dendritic regions of the Purkinje cells (200_{μ} to surface) are but little invaded by impulses (Eccles, Llinás and Sasaki, 1965b) and so act as sources to the deeper sinks produced by the impulses; hence the diminution of these sinks by inhibition would diminish these sources and so increase the negative field potential, particularly at superficial levels. This effect is well illustrated in Fig. 15C by comparison of the inhibited responses with the controls where there appear to be brief positivities on the falling phase that are absent in the most inhibited responses.

With repetitive activation there has invariably been a diminution in potency of climbing fibre synapses. With double stimulation (Fig. 20) this diminution of the second response has the slow time course which characterizes many other synapses (cf. Curtis and Eccles, 1960; Hubbard, 1959, 1963) and which has been attributed (Eccles, 1964) to the slow recovery from the depletion of the immediately available transmitter, although receptor desensitization may also contribute. With repetitive stimulation, the faster the frequency the more rapid and severe is the decrease in the EPSP of the successive response (Fig. 22). As usually observed with synapses, virtual stabilization of the EPSP size occurs after about ten responses. In these respects the climbing fibre synapses exhibit no unusual features. However, the potency of the climbing fibre synapse is such that even with the con-

siderable depression occurring at frequencies as high as 100/sec, each impulse continues to evoke multiple discharges (Fig. 23 E). However, another explanation may be refractoriness of the fine ramifications of the climbing fibres.

The failure of Purkinje cell spikes during still higher frequencies of climbing fibre activation (Fig. 23 F-H) is due not to a deficiency in the EPSP produced by the successive impulses, but to the intense depolarization produced by summation of the successive EPSPs (Fig. 23 I); as a consequence the spike generating mechanism is suppressed, as was originally described by Granit and Phillips (1956). After cessation of the stimulation this depolarization declines with a relatively slow time course (Fig. 23 I). Evidently there is a continuing action of the accumulated transmitter for at least 100 msec after a brief tetanus. As the depolarization gradually declines, spike generation of the soma and dendrites again becomes more and more effective, as indicated by the increasing size of the extracellular spike potentials in Fig. 23 F-H. During these after-discharges there is the expected correlation of increasing size of Purkinje cell spike potentials and slowing of frequency. With more severe tetani after-discharges have been observed to persist for several seconds. Evidently there can be a large accumulation of the excitatory transmitter of climbing fibre synapses, its dissipation being extremely slow. These experiments confirmed Granit and Phillips (1956) in that after prolonged high frequency tetani

Purkinje cells may fire at a high rate for several seconds.

It must be of great significance that in the normal functioning of the cerebellum the single climbing fibres have such an intense excitatory action that, even when firing at a relatively high frequency, each impulse may evoke several discharges from a Purkinje cell as in Fig. 23E. Furthermore, the experiments indicate that a powerful inhibition can succeed only in reducing this frequency of Purkinje cell discharge to one for each climbing fibre impulse, but not in blocking all discharge. Inhibitory action may also be of importance in preventing a high frequency of climbing fibre impulses from effecting an inactivation response of the Purkinje cell, as in Fig. 23. Thus it can be postulated that climbing fibre impulses have a dominating control of the Purkinje cell discharges and that multiple discharges normally evoked by each impulse can be reduced to a varying degree by inhibition, but not blocked. The frequency of Purkinje cell discharge, signals not only the action of the input evoked by its climbing fibre impulse, but also the level of the background excitatory and inhibitory synaptic action on the cell from instant to instant, the excitation being by the parallel fibre synapses, and the inhibition by the basket and stellate cell synapses.

The background excitation by the parallel fibre synapses is controlled by the input via mossy fibres to granule cells to their axons,

the parallel fibres. The negative feedback on this excitatory input, namely parallel fibres to Golgi cells to inhibitory synapses on granule cells (Eccles, Llinás and Sasaki, 1964b), would produce a "disfacilitation" of the Purkinje cells such as has been demonstrated for the cerebellar inhibitory influence on motoneurons (Terzuolo, 1959; Llinás, 1964) and on neurones of the red nucleus (Tsukahara, Toyama, Kosaka and Udo, 1965). Thus parallel fibre stimulation by exciting Golgi cells would remove the background excitatory effect that is produced on Purkinje cells by the mossy fibre-granule cell parallel fibre pathway, and thus exert a hyperpolarizing influence on Purkinje cells. Such a hyperpolarization would be accompanied by an increase of the over-all resistance of the postsynaptic membrane (Llinás, 1964) and thus would aid in explaining the increased size of the CF-EPSP when superimposed on the hyperpolarization produced a parallel fibre volley. It has yet to be demonstrated to what extent the hyperpolarizations produced by parallel fibre stimulation (Andersen, Eccles and Voorhoeve, 1964) are due to this disfacilitation and not to the generation of an IPSP by inhibitory synapses.

IV A) THE FUNCTIONAL ORGANIZATION OF THE CEREBELLAR GLOMERULUS

The granule layer of the cerebellar cortex is one of the most densely packed structures in the central nervous system, there being 3×10^6 granule cells per mm.³ of granule layer, and an overall total

of 10^{10} to 10^{11} cells for the entire cerebellar cortex (Braitenberg and Atwood, 1958). On the other hand, the regularity and simplicity of the synaptic organization of this cerebellar layer is most striking. Basically, the granule layer consists of three types of nerve elements (Ramón y Cajal, 1911; Fox, 1962; Szentágothai, 1965). (i) The small granule cells, 4 to 5 μ in diameter which have four or five short dendrites and one axon directed to the molecular layer, where it bifurcates to form the parallel fibres. (ii) The mossy fibres which are the principal afferent system to the cerebellar cortex, and are the mode of termination of most cerebellar afferent systems, the only known exception being the climbing fibres of the olivo-cerebellar pathway (Szentágothai and Rajkovits, 1959; Eccles, Llinás and Sasaki, 1965a). These mossy fibres end exclusively within the granule layer and are in monosynaptic contact with the dendrites of the granule cells (Ramón y Cajal, 1911). A given granule cell receives inputs from several mossy terminals, on the average about four; and a given mossy fibre may in turn contact many thousands of granule cells. (iii) The large stellate cells, or Golgi cells (Golgi, 1886), which seem to be numerically slightly fewer than the Purkinje cell population (C.A. Fox, personal communication). These cells have a large dendritic tree which ramifies in the molecular layer, and a short axon restricted to the granule layer, which resolves itself by profusely branching to form a very thick plexus and enters into contact with thousands of granule

cells (Ramón y Cajal, 1911; Fox 1962; Szentagothai, 1965).

These three elements are assembled into a single anatomical unit, first described by Ramón y Cajal (1888) and named by Held (1898) the "glomerulus". Although Cajal's description of the glomerulus is essentially correct, only comparatively recently has the actual structure of the glomerulus been thoroughly understood. Electron microscopic studies have shown (Hámori, 1964, Szentágothai, 1965; C. A. Fox, personal communication), that the cerebellar glomerulus (Fig. 24) is a nodular structure formed by the axo-dendritic synapse between the expanded terminal convolutions of the mossy fibres, the claw-like endings of the granule cell dendrites, and the fine axo-dendritic synapses between the axon terminals of the Golgi cells and the dendrites of these same granule cells. These authors showed that interrupting the cerebellar white matter produced a degeneration of the mossy fibres. Under these conditions the synaptic relationship of the Golgi cell axon terminals within the cerebellar glomerulus can be studied. It was indeed found that these axons enter into direct contact with the dendrites of the granule cells. The glomerulus is, thus, a cluster in which two types of presynaptic fibre enter into a complex relationship with one postsynaptic element. The present chapter gives an account of experimental investigations which demonstrate the excitatory character of the mossy fibre granule cell relay (M.G.R.), and the inhibitory action of

the Golgi cell terminals on the dendrites of the granule cells. The Golgi cell functions, therefore, as a negative feed-back to the M.G.R., the main excitatory input to the Golgi cells being the parallel fibres that are seen in transverse section at PF in Fig. 24.

It has been known from early anatomical studies that the granule layer, the deepest of the cortical layers, lies under the layer of Purkinje cell bodies, and extends from 400 μ to about 600 μ below the surface of the cerebellar cortex. In accordance with the anatomical picture, the field analysis of the potentials in the cerebellar cortex has been shown to be correlatable with the activity of the different cerebellar layers. In order to investigate the M.G.R. it is convenient to activate the mossy fibre pathway relatively near to the recording site, in order to avoid temporal and spatial dispersion and the resulting complication of the field potentials; hence they have been stimulated in the white matter of the cerebellum in the vicinity of the fastigial nucleus. This juxta-fastigial (J.F.) stimulation technique, unfortunately, activates not only mossy fibres but also climbing fibres and the axons of Purkinje cells (Granit and Phillips, 1956; Eccles, 1965b). In order to avoid the super-position of field potentials produced by the three different inputs into the cortex, a second stimulation technique was devised, which involves the direct stimulation of mossy fibres by a surface electrode applied to an ad-

jacent folium (Fig. 2). This type of transfolial stimulation (henceforth T.F.) is based on the well known fact that the mossy fibres branch out in the white matter near the surface of the cerebellum, thus producing collaterals which ramify in adjacent folia (Ramón y Cajal, 1911, Fig. 41). Alternate stimulation through the J.F. and T.F. electrodes allowed a direct comparison of their fields in the same folium.

a) Field potentials generated by mossy fibre-granule cell activation.

At levels from 300_u to 500_u depth, the field potential evoked by a single J.F. stimulation (Fig. 25 C-E) consisted of an initial positive-negative (N₁) potential followed by a slower negative (N₂)-positive wave. The initial positive-negative sequence is due in large part to the antidromic activation of the Purkinje cells. The second negativity (N₂) had an average latency of 1.8 msec and an average duration of 2.0 msec with traces of a double summit. From the maximum at about 500_u depth, there was a progressive decline at more superficial levels and eventual disappearance at about 200_u depth. At levels above 200_u (A) there was always a negative wave (N₃) with an average latency of 3.5 msec, and a maximum at a depth of 150_u to 180_u; it rapidly declined at more superficial and at deeper levels.

Fig. 25K-O illustrates the field potentials evoked by T.F stimula-

tion in another experiment. The stimulus artifact concealed a small initial negative wave (n_1) hence the later negative wave which can be seen in Fig. 25 M-O is labelled n_2 , and it resembled N_2 in Fig. 25 C-E in that from its maximum at 500_{μ} it progressively decremented towards the surface to disappear between 300_{μ} and 200_{μ} . However, in this experiment n_2 differed from the usual simple configuration in that it was complex, being split into two by a large sharp positive deflection. At more superficial levels these waves were replaced by the large negative wave (n_3) of Fig. 25 K, L which appears to be homologous with N_3 of the J. F. response (A), both reaching a maximum at about 180_{μ} .

The similarities between these potentials became more evident when they were preceded by a parallel fibre volley set up stimulation of the surface of the cerebellar cortex (LOC) through a concentric electrode. Under these conditions the N_1 wave in Fig. 25 G-J was greatly depressed, signifying that the antidromic invasion of many Purkinje cells was blocked by the inhibitory action of the interneurons of the molecular layer that is the basket and stellate cells. In addition to the inhibition of the antidromic invasion, Fig. 25 shows in F a large depression of the N_3 response and in P and Q of the corresponding T.F. response (n_3). On the other hand at levels of 300_{μ} to 500_{μ} there was in H-J little change in the N_2 response, but a large depression of the subsequent positivity, and in R-T the initial phase of the corresponding n_2 wave was also almost unchanged, but there was a large depression of

the second component of n_2 .

It may be inferred from anatomical studies (Ramón y Cajal, 1911) that the climbing fibres do not participate in the transfolial ramification that is so common with the mossy fibres, since each climbing fibre enters into contact with only one Purkinje cell. This conclusion is in good agreement with our finding that climbing fibre activation of Purkinje cells has never been produced by T.F. stimulation. For reasons to be discussed below, the axon collaterals of Purkinje cells cannot participate in the production of the T.F. potential; hence the response to the T.F. stimulus can be assumed to be mediated solely by the mossy fibres which, by a kind of axon reflex to a transfolial ramification, excite granule cells in the folium under study. The early component of the n_2 wave in Fig. 25 M-O must be produced for the most part by the postsynaptic current associated with the granule cell activation of the mossy fibres, since this potential is not reduced, and in some cases is even increased, by a preceding LOC stimulation. A mossy fibre origin is therefore postulated for the homologous potential (N_2) evoked by J.F. stimulation (Fig. 25C-E). The latency (1.8 msec) and the potential profile in depth of the N_2 waves are in complete accord with their postulated production by synaptic excitation of granule cells by mossy fibre impulses.

It can be also postulated that the second component of n_2 with the associated positive waves in Fig. 25M-O is brought about by the ac-

tivity of the granule cell axons on their way to the molecular layer of the cortex. These axons have been shown (Fox, Siegesmund and Dutta, 1964; Hátori and Szentágothai, 1964) to be grouped in bundles containing twenty or more fibres. The triphasic potential could then be related to the source-sink-source fields which are recorded in a volume conductor when a compound action potential is initiated at a certain distance from the recording place, and is conducted past the tip of the recording microelectrode (Lorente de No', 1947). In Fig. 25, O the initial large positivity at 500_{μ} indicates a still deeper origin for the conducted volley, which gives a large potential up to 250_{μ} from the surface. The N_2 wave evoked by J.F. stimulation must likewise be a consequence of mossy fibre activation, but the excited climbing fibres would also contribute to this wave by synaptic activation of Purkinje cells. However, it is often possible to distinguish between the potentials produced by these two afferent pathways.

b) Mossy fibre activation and Golgi inhibition of single granule cells.

Further evidence for the inhibitory action of Golgi cell terminals on the M.G.R. is provided by single cell recording from the granule layer. In Fig. 26A the "spontaneous" spike activity of several granule cells was recorded extracellularly at 600_{μ} depth with a high resistance microelectrode. In B, the spontaneous activity of all these cells was silenced for a period of 100 msec by a single LOC stimulation; in D

there was a similar inhibition (also 100 msec) following a J.F. stimulation, and in C stimulation of the inferior olive (I.O.) had a small inhibitory action which could be evoked by a possible Golgi cell activation through climbing fibre collaterals (Schiebel and Schiebel, 1954).

In those cases in which a clear recording from a single granule cell has been possible (Fig. 26F-H) a clear-cut inhibitory action by LOC stimulation has always been observed. Granule cells are to be recognized by the following criteria: (i) they must lie in the granule cell layer, that is 400 to 700 μ depth, where they are generally firing "spontaneously", though in some instances granule cell activity can be recorded in an otherwise low noise background (Fig. 26E-H); (ii) they do not fire antidromically to a J.F. electrode, nor orthodromically after a LOC stimulation, but in some cases they may be activated antidromically by a LOC stimulation; (iii) these cells respond repetitively to mossy fibre stimulation (Fig. 26E). It is important to remember that strong LOC stimulation can cause direct mossy fibre activation (see below), so granule cells could be confused in the first instance with Golgi cells, as both are in the granule layer. However, a preceding LOC stimulation will produce an increased repetitive response of Golgi cells (Eccles, Llinás and Sasaki, 1965c), and not the inhibition seen in the case of the granule cells. In general the granule cell spikes have the very small amplitude which would be expected for such small cells.

In Fig. 26E-H, a granule cell was activated 7.5 msec after a stimulus to the ipsilateral superficial radial nerve (SR), possibly via the cuneo-cerebellar mossy fibres (Grant, 1962; Holmquist, Oscarsson and Rosen, 1963). The initial rhythmic burst had a frequency of 500 impulses/sec and was completely inhibited 44 msec after a preceding LOC stimulation (Fig. 26F). Fig. 26G and H show progressive stages of recovery from the inhibition, which was still incomplete at 120 msec. These examples illustrate well the feedback action of the Golgi cell system upon the mossy fibre-granule cell relay, and give some idea of the type of response evoked in the granule cells by a single mossy fibre volley.

c) Field potentials evoked on the molecular layer by mossy fibre-granule cell relay (M.G.R.)

The field potential generated in the molecular layer of the cerebellum by T.F. stimulation of the M.G.R. pathway is a negative n_3 and a later positive wave, with n_3 having in Fig. 27G a latency of 1.7 msec and a peak at 2.5 msec. This T.F.-evoked potential reached its maximum amplitude at 150-180 depth, which is the level of greatest synaptic density of the parallel fibre synapses on Purkinje cells. It is therefore postulated that n_3 is generated by the superposition of the currents produced by the associated activities of the parallel fibre impulses, the excitatory synaptic action of the parallel fibres on the

Purkinje cell dendrites, and the action potentials generated in the Purkinje cells following this synaptic activation.

Although in most cases J.F. and T.F. stimulation evoked somewhat different potential field in the molecular layer (Fig. 25), it was not unusual for the fields to be similar (Fig. 27). In such cases it is assumed that, as with the T.F. response, the J.F. response is entirely due to mossy fibres, which having a larger distribution will produce a widespread activation compared with the restricted activation of Purkinje cells by climbing fibres and antidromic impulses (Ramón y Cajal, 1911; Szentágothai and Rajkovits, 1959; Fox, 1962). In Fig. 27A a negative (N_3) and later positive potential was evoked by the J.F. stimulation and recorded with a microelectrode 140_{μ} from the surface, and there was a very similar potential (n_3) and later positivity (Fig. 27G) as a response to T.F. stimulation. Both the N_3 and n_3 negative waves and the positivities which follow them can be completely blocked by a strong conditioning LOC stimulation, which in Fig. 27 was applied 12 msec previously. From B to F, the strength of the LOC stimulus was raised by steps so that in the lowest record there was complete suppression of the (N_3) response. A similar suppression of n_3 is shown in records H-L, the T.F. response being conditioned by LOC stimulations of equivalent strengths. As the LOC stimulus strength was increased, it produced, concomitant with the in-

hibition, a progressively increasing positive wave (P wave) preceded in D, E and J, K by a negativity with superimposed action potentials of Purkinje cells.

In Fig. 27 the first sign of inhibition by a mild LOC stimulus was the reduction of the amplitude of the positive wave and a much smaller reduction of N_3 (records B and H). In C and I, an increase in the strength of the conditioning volley showed that both N_3 and n_3 were composed of a unitary spike of a Purkinje cell riding on the negative wave, as would be expected if they were generated mainly by excitatory synaptic currents. With increasing LOC stimulation there was a progressive decrease in the spike-like component in A-C and G-I, which also reduced both N_3 and n_3 so that in F and L they too, were suppressed. The LOC stimulation must be preventing the activation of the parallel fibres, and thus blocking the production of the fields at the molecular layer. Again, it must be concluded that as with the inhibition of Fig. 25 this inhibition is mediated by the Golgi cells at the granule layer of the cerebellar cortex.

The time course of the Golgi cell inhibition of a T.F. response by a strong LOC stimulation is illustrated in the specimen records of Fig. 28A-L at three different sweep speeds as indicated by the time scales, A, E and I being the control. In Fig. 28M the sizes of the n_3 responses (ordinates) are plotted against the times after the LOC stim-

ulations on a logarithmic scale. Recovery was almost complete at the longest test intervals.

d) Golgi cell inhibition of the Purkinje cell EPSP evoked by activation of the M.G.R.

The activation of parallel fibres evoked through the crossing over synapse i.e. the synapse between the parallel fibres and the dendritic spikes of the Purkinje cells, (Hámori and Szentágothai, 1964; Fox, Siegesmund and Dutta, 1964) EPSPs in Purkinje cells. It would be expected that the excitation of mossy fibres by J.F. or T.F. stimulation would evoke EPSPs of Purkinje cells by exciting the discharge of impulses from granule cells along the parallel fibres.

Fig. 29 illustrates intracellular records of the EPSPs evoked in response to T.F. stimulation. The Purkinje cell did not discharge on account of partial deterioration by the microelectrode impalement.

These records are an example of the blockage of the Purkinje cell EPSPs by a LOC stimulation. It will be noted in Fig. 29A that the T.F.-evoked EPSP was cut short by the IPSP, generated by the inhibitory action of the stellate and basket cells which were excited by the parallel fibre volley. The EPSPs produced by T.F. stimulation were progressively more depressed as the LOC stimulation was increased in strength (records B-F). On the other hand, the EPSPs evoked in the same Purkinje cell by direct parallel fibre stimulation (LOC stimulus)

appeared to be increased by conditioning LOC stimulation (Fig. 29G-L). In H-L there was even some addition of IPSP by the second LOC stimulus, which contrasts with its complete suppression in D-F. The blockage of the synaptic potentials generated by the T.F. stimulation is thus not occurring in the actions of parallel fibre impulses on the Purkinje, basket and stellate cells, and therefore must take place at the cerebellar glomerulus by the Golgi cell feed-back system.

In M, the amplitudes (as ordinates) of the intracellular synaptic potentials evoked by T.F. and LOC stimulation were plotted against the strengths of the conditioning (LOC_1) stimulus in arbitrary units (abscissae). The plotted volumes were measured from a series similar to that illustrated in records A to L, and were evoked in the same cell. The suppression of the T.F.-evoked EPSP (filled circles) began at a LOC_1 stimulus strength of 30, at which the LOC_1 IPSP was already well developed. It seems as if the inhibition of the M.G.R. by Golgi cells has a higher threshold than the IPSP (open circles) generated by basket and stellate cells. Over the same range of conditioning stimuli (30-60) strengths that produced depression and eventual suppression of the T.F.-evoked EPSPs (filled circles) there was a large increase in the EPSP evoked by the LOC_2 stimulus (filled triangles). This apparent increase in EPSP can be attributed to the suppression or occlusion of the underlying IPSP.

Fig. 30 shows intracellular recordings from another Purkinje cell in which the IPSP was reversed by chloride leakage from the impaling microelectrode. Record A illustrates the "synaptic noise" appearing as reversed IPSPs evoked by synaptic bombardment from basket and stellate cells. Such small depolarizing potentials have been identified as reversed IPSPs generated near the Purkinje cell soma by the tonic repetitive discharge of the inhibitory interneurons (Eccles, Llinás and Sasaki, 1965c). In record B, a single LOC stimulation generated an EPSP followed by a small hyperpolarization, and a period of silence (150 msec). There was a similar removal of the background bombardment after the J.F. stimulation (record C). Stimulation of the lateral cuneate nucleus (D) and of the inferior olive (the stronger stimulus, F) also evoked the removal of the background barrage. Note that in C and F the large initial depolarization is attributable to the excitation of the climbing fibre supplying that Purkinje cell, and that in records C, D and F, arrows indicate the spontaneous climbing fibre responses.

e) Mossy fibre "axon reflex"

In Fig. 31A, B, the EPSP produced in a Purkinje cell by a LOC stimulation had two distinct components. In C a preceding LOC stimulation completely removed the early component of this EPSP, record

B being the control LOC response. The second component of the EPSP was however unaffected. A conditioning LOC stimulus always suppressed the early component of the double EPSP produced by LOC stimulation. This effect is exactly comparable to the large depression of the T.F.-evoked EPSP by a conditioning LOC stimulus in Fig. 29K-N; hence it can be presumed that the LOC stimulus directly excited mossy fibres of the folium that by a kind of axon reflex excited the granule cells, which in turn discharged impulses along the parallel fibres and so to the Purkinje cells. A preceding LOC stimulation would block this pathway by activating the Golgi cell feedback on the granule cells, but the EPSP resulting from the direct LOC stimulation of the parallel fibres would be unaffected as in Fig. 31C.

B) DISCUSSION

The elucidation of the functional significance of the cerebellar glomerulus is of great interest in the understanding of the physiology of the central nervous system, since it allows an immediate translation from structure to function. Anatomically the glomerulus is one of the best known neural structures, its morphology having been thoroughly studied at the ultrastructural level (Szentágothai, 1965; Hátori, 1965; Fox, personal communication). When the cerebellar white matter is undercut leaving the cerebellar cortex intact and time

allowed for degeneration of the mossy fibre components of the glomeruli, electron-microscopic examination reveals that axon terminals of the Golgi cell are in direct synaptic contact with the dendrites of the granule cells. This observation implies that the Golgi cell axons exert a postsynaptic inhibitory influence on the granule cell dendrites.

The functional relation between the three elements of the glomerulus can be formulated as follows: the mossy fibres exert a synaptic excitatory action on the granule cells (Fig. 26) and impulses discharged along their axons (the parallel fibres) excite the other cells in the cerebellar cortex including the Golgi cells, the only known exception being the Lugaro cells (Fox, 1963). The axons of the Golgi cells end exclusively in the granule layer as inhibitory synapses on the dendrites of the granule cells. The straightforward negative feed-back is, however, complicated by the fact that the Golgi cell receives excitatory, and possibly inhibitory inputs from other sources. It has been suggested by Szentagothai (1965) that some of the mossy fibres enter into direct contact with dendrites of the Golgi cells in the superficial levels of the granular layer. This direct input would produce a feed-forward type of inhibition from mossy fibres to the granule cells.

In addition to the direct mossy fibre input, anatomists have also described synaptic terminals to Golgi cells from collateral of climbing fibres (Schiebel and Schiebel, 1954; Szentagothai and Rajkovits,

1959). These collaterals could be responsible for the inhibitory action exerted by inferior olive stimulation on the spontaneous activity of granule cells (Figs. 26C and 30F).

The Golgi cell negative feed-back onto the granule cells is much more localized than the inhibitory action of the basket or stellate cells (Szentágothai, 1963, 1965). The Golgi cell axon plexus terminates in the granular layer directly under the molecular layer containing its dendritic tree. This particular arrangement allows the Golgi cell to exert negative feed-back upon the same cells that activate its dendrites, as well as upon all the granule cells under a given activated beam of parallel fibres. Thus it can be regarded as an "on beam" operator, in contrast with the "off-beam" inhibition by basket cells (Szentágothai, 1963, 1965; Andersen, Eccles, Voorhoeve, 1964). The "on beam" operation is the more interesting when the particular distribution of the Golgi cell dendrites is taken into account. The dendrites of these cells, unlike the dendritic trees of all the other cells in the molecular layer, do not have a planar arrangement oriented orthogonally to the direction of the parallel fibres. Finally, the Golgi cell acting as a feedback to the cerebellar glomerulus is in fact regulating the parallel fibre input into the molecular layer, and is thus controlling the background bombardment evoked by the mossy fibres at the granular layer. In view of its large number of elements, the granular layer must function as a phasic to tonic converter in which the

activity of a single mossy fibre is spread through a large area of the cerebellum, sometimes including more than one folium, and so enters into contact with thousands of granule cells, whose impulse discharges in turn propagate along the parallel fibres. Since a given parallel fibre can as a rule only contact the dendrites of a Purkinje cell once, due to their planar arrangement, the granular layer is in fact a divergence mechanism. A given parallel fibre can contact as many as 460 Purkinje cells (Fox and Barnard, 1957) and one Purkinje cell receives from as many as 60,000 to 120,000 parallel fibres and so potentially is able to integrate this extraordinary rich convergence (Fox and Barnard, 1957). Owing to their dendritic impingement the parallel fibres function as a diffuse input, as opposed to the very specific activation of each Purkinje cell by a single climbing fibre. The background barrage that the granular layer impresses upon the Purkinje cells via parallel fibres can be depressed to varying degrees by the inhibitory action of Golgi cells.

V A) THE PARALLEL FIBRE SYSTEM AND ITS FUNCTIONAL RELATIONS WITH THE NEURONES OF THE CEREBELLAR CORTEX.

The parallel fibres in the molecular layer of the cerebellum constitute a remarkable system of neuronal interconnections. They were originally described by Ramón y Cajal (1888, 1911) and shown to arise from the bifurcation of the axons of the granule cells which ascend vertically from the granular layer of the cortex. Fox and Barnard (1957)

and Braitenberg and Atwood (1958) have described in geometrical and numerical terms the synaptic relationship of these fibres to the Purkinje cell dendrites. Electronmicroscopic investigations by Gray (1961), Fox, Siegesmund and Dutta (1964) and Hátori and Szentágothai (1964) have substantiated the suggestion made originally by Ramón y Cajal (1911) and later by Fox and Barnard (1957) that each dendritic spine in the Purkinje cell dendrite receives a synaptic connection by a parallel fibre, the so-called crossing-over synapse. Dendritic spine counts suggested (Fox and Barnard, 1957) that each Purkinje cell receives at least 60,000 synapses from the large number of parallel fibres (200,000 to 300,000) which project through the territory of its dendritic tree. Hátori and Szentágothai (1964) increased the estimate of crossing-over synapses on a given Purkinje cell to 120,000 because they found that on the average each dendritic spine has a secondary spine which normally participates in a synapse, from a different parallel fibre from that supplying its parent. Each parallel fibre probably extends for as long as 1.5 mm in each direction from its origin in the bifurcating granule cell axon (Fox and Barnard, 1957; Hamori and Szentagothai, 1964; Szentagothai, 1964, 1965), and it is estimated that it will traverse the dendritic trees of up to 500 Purkinje cells (Fox and Barnard, 1957). In addition the parallel fibres make crossing-over synaptic contacts with other types of neuronal dendrites in the molecular layer; - those of basket, superficial stellate and Golgi cells.

In this part of the investigation the parallel fibres were directly stimulated through a surface electrode applied to the centre of a folium with a broad surface, and the extracellular field potentials were recorded by a microelectrode inserted vertical to the surface of the folium and in various geometrical relationships to the stimulating electrode (Fig. 32). This procedure follows that originally developed by Dow (1949). Potential profiles along such vertical tracks and also transverse potential contours were employed in the attempt to analyze the observed potentials and to relate them to the neuronal structures of the molecular layer.

a) Field potentials generated by impulse propagation in parallel fibres.

When the parallel fibres are stimulated by a current applied through a LOC. electrode, the very restricted distribution of the applied current would be expected to result in the excitation of a narrow band or beam of parallel fibres (Dow, 1949 ; Andersen, Eccles and Voorhoeve, 1964), because these parallel fibres have been shown to run in the molecular layer with an orientation strictly longitudinal to the folium (Ramón y Cajal, 1911; Fox and Barnard, 1957; Fox, Siegesmund and Dutta, 1964). An appropriately located microelectrode should therefore record the compound action currents generated by conducted action potential in the parallel fibres, as is illustrated in Fig. 32B.

A stimulus about three times threshold strength (100 in lowest trace) evoked a brief triphasic potential followed by a slow negative wave. With progressive reduction of the stimulus strength (80 to 40 in B) there was a corresponding diminution of the triphasic potential, but it retained its configuration and there was only a slight lengthening of latency even for a stimulus just above threshold. The triphasic potential is, of course, characteristic of a fairly well synchronized volley of impulses propagating in a bundle of nerve fibres immersed in a volume conductor, as is the case for the parallel fibres. If the triphasic potential is in fact generated in this way, the sizes of the potentials evoked by the graded stimuli in Fig. 32B correspond approximately to the number of parallel fibres excited by the stimulus.

Comparison of Fig. 32C and D shows that the brief initial potential declines in depth much more rapidly for the weak LOC stimulus (C, 60 strength) than with the strong stimulus (D, 100 strength). Correspondingly also the later slow negative wave reverses to a positive wave much more superficially (at 0 to 30_u) in C than in D (60 to 80_u).

It is postulated that triphasic potentials of Figs. 32 B and D are directly produced by the volley of impulses in the parallel fibres. This postulate has been tested by the several procedures described below. These tests render untenable an alternative postulate that the major part of the negative phase of the triphasic potential is generated by the Purkinje cells in response to the excitatory synaptic ac-

tion of the impulses in the parallel fibres. (Andersen, Eccles and Voorhoeve, 1964). The potentials produced by this synaptic excitatory action will be considered later.

b) Conduction times along the beam of excited parallel fibres

For the series of Fig. 33A, a stimulating current of three times threshold was applied through a LOC electrode in a fixed position on the folium. By searching with the recording microelectrode it was established that field potentials with a negative spike-like component could be recorded only in a narrow band running longitudinally along the folium. The potentials along this band (Fig. 33A) are arranged in this figure downwards in their order of recording, the distances being expressed relative to the position of closest apposition of the recording electrode. This termination of impulses after various distances of to the concentric stimulating electrode. Since the over-all diameter of the stimulating electrode was almost 1 mm and the central core was the cathode, a conduction distance of rather more than 500μ should be added to the distance indicated in Fig. 33A in order to obtain the actual conduction distances. From the progressively increasing conduction times, a conduction velocity can be calculated as shown in the approximately linear relationship for the plotted points of Fig. 33B. Measurements from the bottom of the initial positive wave (filled circles) give a velocity of about 0.39 m/sec for the fastest impulses

of the volley, while a velocity of about 0.33m/sec is given for the crests of the negative potentials (filled triangles). These values are in good agreement with the value of 0.3 to 0.5 m/sec obtained by Dow (1949) using a similar technique.

Besides the progressively longer latent period, the field potentials of this figure show several other features which are in accord with their postulated derivation from conducted impulses. There is firstly the progressive decrease in height and increase in duration, which would be expected for a volley of impulses becoming more dispersed in time during conduction. Since, as stated above, the total length of the parallel fibres may be no more than 3 mm, the diminution of field size will also arise on account of the termination of many of the excited fibres at various distances from the stimulating electrode. This termination of impulses after various distances of conduction is also indicated by the progressive decline, and the eventual extinction beyond 700 μ of the third phase of the triphasic field potential. The terminal positive phase is, of course, dependent on the propagation of the impulses well beyond the recording electrode (Lorente de No', 1947).

Investigations on the impulse propagation along parallel fibres by the technique of direct cortical stimulation, as in Fig. 33, are often complicated by the direct stimulation of mossy fibres in the granular layer.

The impulses generated in this manner will then spread through the widely branching mossy fibres (Ramón y Cajal, 1911) and in turn excite granule cells which through their axons, the parallel fibres, will synaptically activate Purkinje cells. By this "indirect pathway" large potentials can be evoked in the molecular layer, which, on account of the relatively fast propagation in the mossy fibre branches, precede the arrival of the impulse directly propagating along the parallel fibres for more than about 0.5 mm. Thus in Fig. 33A the arrows mark potentials produced in this manner, which are quite small at that superficial level of recording, but which became large when recorded at a depth of 40_{μ} to 80_{μ} . For example the arrows in C mark the onsets at conduction distances of 1700 to 700_{μ} . When the stimulus was reduced to 60 strength, D, the complications attributable to mossy fibre excitation were much diminished, though still relatively large in the upper two traces, and at the same time the responses at depths of 40_{μ} to 60_{μ} were almost pure positive waves. The mode of production of this wave will be considered below, but for the present its onset at the different conduction distances (arrows in D) can be regarded as an alternative measure of the respective conduction times. The points as plotted in Fig. 33B (open circles) are seen to give much the same conduction velocity for the parallel fibres, about 0.39 m/sec.

It can thus be stated that this figure provides an illustration of

the ways in which the conduction velocity of parallel fibres can be measured and gives an example of the complication produced by direct mossy fibre stimulation. There have been only a few measurements of parallel fibre conduction velocity, the mean value being 0.30 m/sec for the fastest propagation (as with filled circles B). This velocity is in good accord with the diameters of the largest of the superficial parallel fibres, which is 0.2 to 0.3_u (Fox, Siegesmund and Dutta, 1964; Hátori and Szentágothai, 1964).

The complications introduced by stimulation of mossy fibres as seen in A and D, can be eliminated by performing this experiment on the chronically deafferented cerebellum. Surface stimulation was routinely employed in many investigations, there being in all experiments the triphasic responses attributed to parallel fibres, as well as the later slow potentials. No trace was ever observed of the potentials of Fig. 33 C and D, which were ascribed to mossy fibre stimulation. These experiments thus fully corroborate the postulates that these triphasic potentials are produced by impulse conduction in the parallel fibres, and that the earlier potentials in C and D are secondary to the excitation of mossy fibres.

c) Experiments on the refractoriness following a parallel fibre volley

As shown schematically, in Fig. 34, two stimuli of identical strength were applied through the same LOC. electrode and the re-

sponses were recorded by a microelectrode inserted to a depth of 30_{μ} in the excited beam of parallel fibres. With stimulus intervals down to 3.4 msec the second stimulus evoked a full-sized field potential and the second volley contributed a large addition to the slow negative potential generated by the first volley as seen in the superimposed traces. This positive potential and its relation to the early negativity will be considered later. At the moment it is sufficient to note that the second sharp negative potential was progressively diminished with shortening of the stimulus interval, so that it was greatly reduced in size at 1.1 msec. At 0.8 msec there was still a small addition to the declining phase of the negativity and of the slow negative wave, while at 0.6 msec the second stimulus was quite ineffective. It is thus possible to demonstrate that the early negativity of Figs. 32, 33 and 34 has an associated refractory state, exactly as would be expected for spike potentials produced by impulses propagating in the parallel fibres. There have been a number of similar series with double stimuli in several experiments.

An alternative method of demonstrating the refractoriness associated with the propagated spike potentials in the molecular layer is shown in the schematic diagram of Fig. 35. Stimulation was applied through two LOC electrodes which were carefully adjusted in the longitudinal axis of the folium so that they excited the same band

of parallel fibres (cf. Fig. 32A). The recording microelectrode was inserted at a depth of 30_{μ} into this excited band at approximately the midpoint (cross in diagram of Fig. 35) between the two LOC electrodes. At the longest stimulating interval (11.2 msec) the second stimulus evoked a full-sized early negativity followed by a large negative wave, just as in Fig. 34 at intervals of 3.4 to 9.2 msec. As the stimulus interval was shortened from 11.2 to 5.7 msec, there was progressive decrease in the negative potential. The added negative wave was negligible at 7.3 msec; and there was actually a reverse (positive) slow potential at 5.7 msec. At the still shorter stimulus intervals, there was no appreciable change in the early negativity; and, so far as can be judged in the absence of superimposed traces the after-potential added by the second stimulus was changed but little.

Many similar series of observations were made in this and in other experiments with either stimulus leading the other. A satisfactory explanation for those findings is readily provided in terms of the refractoriness following an impulse and of the annihilation of impulses by collision. Stimulation through electrode 2 can be fully effective in evoking a parallel fibre volley (as at 11.2 msec in Fig. 35) only at the end of the refractoriness which follows the impulses which travel from LOC 1 to LOC 2. Measurements of the negative potential from its origin in the depth of the initial positive wave,

show that the respective conduction times from LOC 1 and 2 to the recording electrode were 3.2 and 1.9 msec respectively, giving a conduction time of 5.1 msec from LOC 1 and 2. At briefer intervals than 5.1 msec there would be collision of the majority of the impulses. At the slightly longer intervals (7.3 and 8.1 msec) the effectiveness of the stimulation at 2 would be depressed by refractoriness just as in Fig. 34. However, this depression would be incomplete because, as indicated in the diagram, many of the parallel fibres excited by LOC 2 would not extend beyond the recording electrode to LOC 1. Such parallel fibres could not be excited by the initial stimulation at 1, and so were available for excitation by LOC 2 at all test intervals. The volley in this group of fibres evoked the field potentials of approximately constant size at test intervals of 5.7 msec and briefer in Fig. 35. It is evident that the experimental observations are in precise accord with the postulate that the sharp negative field potentials are generated by the propagation of impulses in the parallel fibres.

d) The slow potential waves produced by a parallel fibre volley

In Figs. 32 to 35 LOC stimulation evoked slow potential waves in addition to the early potential attributable to the impulses in the parallel fibres. A preliminary account of these waves has already been given (Andersen, Eccles and Voorhoeve, 1964). Since then larger

series of investigations have been performed on these slow potential waves in an attempt to elucidate problems concerned with their mode of production. Recording at various depths and also transversely in the plane orthogonal to the excited beam of parallel fibres revealed a complex pattern of wave forms, which was not susceptible to any simple interpretation. The complications arising from the stimulation of mossy fibres (Fig. 33C,D), were not appreciated in the preliminary account and have since been eliminated by utilizing the chronically deafferented cerebellum. Possible complications from stimulation of climbing fibres were likewise eliminated by this procedure.

Fig. 36 illustrates the potentials recorded from a cerebellum completely deafferented by bilateral section of all six peduncles 22 days previously, and so effectively degenerating all climbing and mossy fibres (Hátori, Szentágothai, 1965, Fox, personal communications). In this experiment the LOC stimulus was about 1.5 times threshold, and its effectiveness was monitored throughout the whole experiment by surface recording of the parallel field spike potential, as may be seen in the lowest traces of the columns of Fig. 36. Each column shows the potentials evoked by the LOC. stimulus at the indicated depths. In order to ensure maximum reliability for these depths, as a routine procedure the recordings were made during step-wise withdrawals of the microelectrode, which in each

track was initially inserted to a depth of 100_{μ} beyond that used for the deepest recording. The tracks of Fig. 36 were in the temporal sequence A, B, C, D. Additional tracks with the indicated lateralities E (100_{μ}), F (300_{μ}) and G (500_{μ}) were taken but are not illustrated. They were in general appropriately transitional (cf. Fig. 37B), the responses of track E (100_{μ}) for example lying between those of A and D.

In track A the initial field potentials recorded at depths of 0 to 100_{μ} (Fig. 36) show that the microelectrode was inserted accurately in the middle of the beam of excited parallel fibres. There was still a considerable initial negativity in the same superficial recordings at 200_{μ} transversely across the folium (track D); but not at 200_{μ} further still (track C). At depths of 0 to 50_{μ} in track A the initial diphasic potential (positive-negative) declined into a slowly decaying negative potential which had a duration of at least 20 msec. However, at 100_{μ} depth the small initial fast potential was followed by a positive potential which was virtually a mirror image of the slow negative potential at 50_{μ} . At 200_{μ} depth an even larger slow positive wave followed the initial small negative component. The slow positive wave rapidly diminished in size with increasing depths from 300_{μ} to 600_{μ} .

In general the potentials of track D (200_{μ}) resembled those of track A, but the initial diphasic fast potential and the associated slow

negative wave were smaller, and reversal occurred more superficially (at 50_{μ}), while the slow positive wave was considerably larger at 200_{μ} and deeper thereto. In track C (400_{μ}) this tendency for the positive wave to be larger in depth is further exemplified at 300_{μ} and deeper thereto. However, in track B (600_{μ}) the positive waves were small at all depths.

Since the declining phases of the slow negative and positive waves had comparable time courses throughout the whole series of Fig. 36, it is convenient to compare the sizes by measuring all potentials at the same fixed interval after the LOC. stimulus. The interval of 5.3 msec was chosen because it was long enough to avoid complications due to spike potentials of the parallel fibres, and short enough for the measurements to be made just after the maximum of most potentials. This interval is shown in Fig. 36 as the vertical broken lines. The measurements give the plotted potential profiles of Fig. 37A. There were essentially similar potential profiles, but of lower voltage, with measurements made at intervals up to 10 msec after the stimulus. The potential profiles on beam and 200_{μ} lateral thereto had a very sharp negative to positive transition at depths up to 100_{μ} ; this was also the case with Track E at 100_{μ} lateral. Evidently a large extracellular current was flowing from sources deeper than 100_{μ} to superficial sinks. Deeper than 200_{μ} there were in these three tracks less steep potential profiles in the reverse sense,

indicating an extracellular current from sources about 200_u depth to still deeper sinks. However, at Track C (400_u lateral) and to a lesser extent in tracks at 300_u and 500_u lateral, the source was deeper; and in track C the potential profile from positive to negative became steep only at depths beyond 400_u . In Fig. 37A, track B, it will be seen that the positive potential waves were almost identical from 150_u to 400_u depth, which indicates that there was a fairly uniform source over this range of depths with sinks superficial and deep thereto.

This derivation of extracellular flow from potential profiles is, of course, approximate and would be accurate only if similar profiles occurred at that particular instant in all parallel tracks in that region. The deviation from vertical uniformity can be recognized in the contour field plot (Fig. 37B) for all seven tracks (A-G). From 0 to 400_u laterally it can be seen that at depths below 150_u this uniformity was approximately obtained, and it can be assumed that over this range the current flows were approximately vertical within the column sampled by each track. However, at more superficial levels strong currents would be flowing obliquely from deeper lateral sources to superficial medial sinks; and lateral to 400_u there was a steep gradient giving laterally directed currents from sources concentrated at depths of 150_u to 400_u in the column sampled by track C. On account of the relatively long extension of the

parallel fibre volley along the folium, it can be assumed that similar transverse profiles would be observed even at distances as far as 500_{μ} on either side of that depicted in Fig. 37, i.e. that the band of excited parallel fibres evoked a potential field having much the same transverse contour for a considerable distance along the folium. No attempt has been made to test systematically this inference from structural design, but in many experiments parallel fibre volleys were observed to set up similar potential fields at a series of positions along the folium.

In three other experiments on chronically deafferented cerebella, LOC. stimulation set up similar potential profiles. In two of these a number of tracks in a transverse plane were also carefully studied and showed potential contours having a general resemblance to that of Fig. 37. For example in one experiment the maximum positive wave was recorded at a depth of 200 to 250_{μ} and at least 200_{μ} lateral to the beam of excited parallel fibres; and as in Fig. 37 the positive wave was still quite large at 400_{μ} lateral to the beam and at a depth of 400_{μ} .

Examples have already been published (Andersen, Eccles and Voorhoeve, 1964, Figs. 4, 5) of the potential profiles generated by LOC. stimulation of a normal cerebellar folium. Unfortunately, the field potentials were however complicated by the direct excitation of mossy fibres in addition to the parallel fibre system, and at depths

of 0.4 to 0.55 mm large responses of granule cells evoked via this mossy fibre excitation can be seen in Fig. 4 of that paper. Fig. 38 gives the most detailed contour map of the potential field generated by a parallel fibre volley. Though the strength of stimulation was only 1.7 times threshold for the parallel fibres, there were quite large responses due to mossy fibre stimulation, particularly at about 300_{μ} lateral to the beam. Mossy fibre stimulation was much less prominent at a strength of 1.5 times threshold, but the contour map had in general a similar configuration, though at a lower voltage (Fig. 38B). In both of these contour maps an important feature was the relatively large size of the positive waves both deep and lateral to the superficial focus of maximum positivity. The lateral extension was greatest at depths from 200 to 300_{μ} .

e) Potential waves evoked by a second parallel fibre volley at various intervals

In order to avoid complications from the inadvertent stimulation of climbing and mossy fibres, the experimental investigation has been carried out on four chronically deafferented cerebella. The initial illustration will be drawn from these experiments, but it will appear later that essentially the same responses occur in the many experiments on the normal cerebellum.

In Fig. 39A-F, the recording microelectrode was very superficial (10_{μ} depth) and in the excited beam of parallel fibres, as may be seen

by the initial positive-negative spike potential. A second similar stimulus was applied through the same LOC. electrode at various intervals in A-E, F giving the control response. In responses C to E there was a clear potentiation of the slow negative wave produced by the conditioning stimulus. The sizes of this wave at 4.5 msec after the stimulus are seen to increase almost to double the control value with intervals of 5 to 15 msec. There was still a considerable potentiation at 26 msec. Throughout all this period of potentiation, the initial diphasic wave was slightly subnormal; hence the potentiation cannot be attributed to an increased effectiveness in the excitation of parallel fibres by the applied stimulus.

At a depth of 50_{μ} there was also potentiation of the negative wave (Fig. 39G,H), but it was much less prominent in the control (I) than at 10_{μ} , and actually there was reversal to a slow positive wave deeper than 50_{μ} . For example, at 100_{μ} (J,K) the positive wave immediately followed the initial spike and was depressed to about 75% over the same range of intervals that gave the potentiation of the superficially recorded negative wave. A slight depression was also observed for the second positive wave at depths of 150_{μ} (L,M) and 200_{μ} (N,O). In contrast, the second positive wave was a little potentiated when observed in tracks 200_{μ} to 400_{μ} lateral to the excited beam. In part this apparent potentiation was due to inhibition of the spike discharges of Purkinje cells which occurred as

brief negative deflections during the depth of the positive wave.

Fig. 40A, B, illustrates in another deafferented cerebellum the transitional stages between the superficially recorded slow negative wave with potentiation of the conditioned response evoked by a second LOC. stimulus at depths of 0 and 30_u , and at depths of 100 to 200_u the virtually mirror-image positive wave with a slight depression of the conditioned response. At the intermediate depths of 50_u and 70_u there was potentiation of the negative wave, but at 70_u it was submerged by the positive wave, and a submerged trace can also be detected at 100_u depth (arrow). A comparable potentiation of the slow negative wave occurred in the other two chronically deafferented cerebella.

Potentiation of the superficially recorded negative wave was also a regular feature of normal cerebella. For example in Fig. 34, at stimulus intervals of 3.4 to 9.2 msec, the second parallel fibre volley added to the first response a negative potential considerably larger than it evoked in the control response.

Similarly, in Fig. 35 the second volley added a quite large slow negative wave at stimulus intervals of 9.0 and 11.2 msec, and even at 8.1 msec there was addition of a negative wave, whereas in the control response there were merely two brief negative potentials (at arrows) which probably signal the production of impulse discharges in Purkinje cells. In contrast, at intervals of 5.7 msec and briefer

the second volley actually effected a removal of some of the slow negative wave generated by the conditioning stimulus. It is important to keep in mind that this response is due to the volley of parallel fibre impulses which survived the collision and hence must be in different parallel fibres from the volley which gave the conditioning response. Thus there would be no opportunity for the potentiation which occurs in the production of the slow negative potential by a second volley in the same parallel fibres as in the first (Figs. 34, 39A-E, 40B). Presumably, the relative positivity actually produced by the second volley at intervals of 2.9 to 5.7 msec in Fig. 35 is attributable to the slightly off-beam position of the recording electrode relative to the parallel fibre volley which survived the collision, as for example may be seen at quite superficial levels in Figs. 32 C, 33D, 36D. This production of a relatively positive slow wave by the volley surviving the collision was also observed in several other experiments.

Only in two experiments was the potentiation of the slow negative wave studied at longer intervals than those so far illustrated. In these experiments it was still detectable, but of reduced size at intervals of 60 msec and 90 msec respectively.

In Figs. 39K, M, O, and 40B the positive wave recorded at a depth of 100_{μ} or more in the molecular layer was depressed at brief

intervals after the conditioning parallel fibre volley. As at 100_{μ} in Fig. 40B this effect was clearly due to the antagonism of the potentiated slow negative wave, but in other cases it appeared as if there was a genuine depression in the production of the positive wave itself, and depressions have been observed with testing intervals in excess of 100 msec in the experiment illustrated in D, for a testing interval of about 20 msec, and 250 msec was required for full recovery in another experiment. On the other hand, in some experiments there was a potentiation of the positive wave, as for instance at depths of 150 to 400_{μ} in C. This potentiation has been observed for testing intervals as long as 60 msec.

f) Generation of impulses in Purkinje cells by the parallel fibre volleys

In a later section there will be an account of the intracellular recording of the spike potentials which a parallel fibre volley evokes in Purkinje cells. These spike potentials are also observed with extracellular recording and examples have already been published (Eccles, Llinas and Sasaki, 1964a). These responses have the latency to be expected if they were produced by the direct excitatory action of the parallel fibre volley on the Purkinje cells.

In Fig. 41A, stimulation by the LOC. electrode evoked a single spike potential recorded at a depth of 250_{μ} , the latency rang-

ing from 2.5 to 3.7 msec. A second similar LOC. stimulus also evoked a spike potential when the stimulus interval was between 0.9 and 2.8 msec. At longer intervals up to 10.3 msec (A) the second volley always failed to evoke a discharge, and slower recording showed that the upper duration for this suppression was 50 msec.

The unitary response in (A) indubitably was produced by a Purkinje cell. The only other cells at that level (250_{μ} depth) of the molecular layer would be basket and stellate cells. In both of these types a single parallel fibre volley evokes a repetitive discharge, and with two volleys in succession there would be a potentiation of the response to the second volley with an increased number of discharges, not the inhibition observed here. This inhibition is characteristic of Purkinje cells and is attributable to the postsynaptic inhibitory action of the basket and stellate cells that are directly excited by the conditioning parallel fibre volley (Andersen, Eccles and Voorhoeve, 1964; Eccles, Llinás and Sasaki, 1965c, 1965d). Presumably it is the early onset of this inhibition that precludes the repetitive discharge of Purkinje cells, despite the long duration of the synaptic excitatory action evoked by a single parallel fibre volley (Eccles, Llinás and Sasaki, 1965b). In Fig. 41A, 7.0 msec was the longest time after the conditioning stimulus at which the second volley could evoke a discharge. Despite the potentiation of

the excitatory synaptic action produced by the second volley and its summation with the residual action produced by the first volley, the inhibition was completely effective with stimulus intervals from 3.3 msec to 50 msec.

Fig. 41B gives an example of the generation of a Purkinje cell discharge by a parallel fibre volley in a chronically deafferented cerebellum. It appears that at least two Purkinje cells were responding to the stronger stimuli, and the latencies of the responses were shortened by strengthening the stimulation. In C the response to a large parallel fibre volley was inhibited by a similar conditioning volley at intervals of 10 to 20 msec. However, the inhibition was only partial, probably of one cell, and not total as in A.

In Fig. 11D the conditioning LOC. stimulus did not evoke a discharge from the Purkinje cell under observation, but it inhibited the discharge evoked by a different LOC. electrode with stimulus intervals of 2.5 and 6.3 msec and sometimes of 7.3 msec. The conditioning volley here was causing the inhibition of a Purkinje cell which was too far off-beam to be excited, an effect which is to be expected for the basket and superficial stellate cells with their laterally directed axons (Ramón y Cajal, 1911; Szentágothai, 1963, 1964, 1965).

Sometimes superimposed on the slow negative potential that follows the parallel fibre spike there were one or two small spike-like

potentials. For example, they occurred in the CON traces in Figs. 34 and 35, and were inhibited by the preceding LOC. stimulation even when the second LOC. stimulation then evoked an increased slow negative wave. This inhibition parallels that illustrated in Fig. 41.

B) Parallel fibre activation of the interneurons of the cerebellar cortex

It is known since Ramón y Cajal that the parallel fibres establish "crossing-over" synapses with the dendritic spines of cells in addition to the Purkinje cells in the molecular layer of the cerebellar cortex (Ramón y Cajal, 1911; Fox, 1962; Szentágothai, 1964b; Hátori and Szentágothai, 1964a). It seems reasonable to assume that these synapses on the dendritic spines of the basket, superficial stellate and Golgi cells are all similar in action to the excitatory synapses made by these same parallel fibres on the dendritic spines of Purkinje cells (Andersen, Eccles and Voorhoeve, 1964; Eccles, Llinás and Sasaki, 1964b). Furthermore, there is evidence that two different kinds of fibres, probably axon collaterals from Purkinje cells and from climbing fibres, make synapses on the somata and dendrites of these three types of cells (Schiebel and Schiebel, 1954; Szentágothai, 1964a; 1964b; Hátori and Szentágothai, 1964a, 1964b). There is experimental evidence suggesting an inhibitory function for basket cells (Andersen, Eccles and Voorhoeve, 1964), for Golgi cells (Eccles, Llinás and Sasaki, 1964b) and for the superficial

stellate cells. As shown in Fig. 2 the axons of these three types of presumed inhibitory cells (BC, GoC and SC) terminate within the cerebellar cortex at not more than a few hundred microns from their respective cell bodies (Ramón y Cajal, 1911; Jakob, 1928; Fox, 1962; Szentágothai, 1964b).

It will be convenient to refer to these cells collectively as the interneurons of the cerebellar cortex, ignoring in this terminology the other cortical interneurons, (the granule cells and the Lugaro cells). The only criterion for discriminating between the three types of interneurons under present consideration is provided by the depth below the surface of the cortex at which the sharply localized spike potentials can be recorded, it being assumed that this gives the approximate level of the respective somata. According to anatomical evidence (cf. Fig. 2) the somata of the Golgi cells are almost always situated just deeper than the layer of Purkinje cell bodies, i.e. at about 400 to 500_μ, while just superficial thereto are the basket cell somata, i.e. at about 300 to 350_μ. The somata of the stellate cells are still more superficial. A classification of the presently investigated cells according to the depth criterion reveals that no criterion for discrimination is immediately obvious in their responses to the synaptic inputs here employed. It is therefore simplest to describe the responses to these various synaptic inputs for all interneurons regardless of their cortical level, and then in

the discussion to examine the relationship between cortical level and the observed responses.

a) Single parallel fibre volleys

A typical series of responses of an interneurone of the cerebellar cortex is illustrated in Fig. 43. The large extracellularly recorded spike potentials are always diphasic with an initial positivity, which is the typical configuration when an electrode is in very close proximity to a neurone. A weak stimulus applied to the parallel fibres evoked a single response with a latency of 5.5 msec (A), and with progressively stronger stimulation there was an increase in the number of spike discharges and a shortening of latency, which was only 1.5 msec with the stronger stimulations (D to G). The spike discharges were as close as 2 msec apart in F and G and the total duration of the discharge was in excess of 30 msec. The predominantly negative wave in the lower traces was recorded by a surface electrode which was considerably further from the stimulating electrode than the recording microelectrode, and not closer as with SRE in Fig. 2, and is produced as has been seen above by the propagated action potentials in the parallel fibres.

The differentiation between Purkinje cells and interneurones can be made on the following basis:

In the case of Purkinje cells there is usually only one or two

spike discharges even to a large parallel fibre volley, the discharge being curtailed by the inhibitory action of the interneurons of the molecular layer. The response to juxta-fastigial stimulation also gave a clear-cut discrimination from Purkinje cells. Instead of the antidromic spike potential with a latency usually of 0.6 to 1.0 msec (Figs. 4C; 8; 10); there was in Fig. 43J a more delayed spike potential, the latency being 2.4 msec. Its production by synaptic activation of the cell was demonstrated by the variability in its latency and by its failure to follow high stimulus frequencies, the following of stimulus frequency being often no higher than 10 msec.

Close inspection of the rhythmic responses of Fig. 43D-G reveals that with the stronger stimulations the second cycle may be briefer than the first. During the high frequency response there was a reduction in size of the spike potentials. Undoubtedly refractoriness contributes to this reduction; but to a considerable extent it must be accountable to the continued severe depolarization that generates the high frequency discharge, just as has been observed with the synaptic excitatory action of climbing fibres on Purkinje cells

The repeatable character of the discharge evoked by a given parallel fibre volley is illustrated by the superimposed tracings in H and I. As a first approximation it can be postulated that at any instant the reciprocal of the rhythmic cycle (the frequency of the discharge) is a measure of the intensity of the excitatory synaptic

transmitter action. In K the frequency thus calculated is plotted against time after the stimulus so as to give an approximate time course of the excitatory transmitter action. A tendency for an increase during the first two cycles of the responses to the stronger stimuli was seen, which is in contrast to the similarly plotted curves for the synaptic activation of Renshaw cells, where the first cycle was always the briefest (Eccles, Eccles, Iggo and Lundberg, 1961). There is also the suggestion in K that the time course of the transmitter action was similar for the weak and strong stimulations. Presumably the effect of stronger stimulation in shortening the latency in A to D was in part due to the steeper rise of transmitter action, though doubtless the shortening of neural conduction time by the spread of stimulation along the parallel fibres was a contributory factor. However, measurements of the latencies of the parallel fibre spike potentials in the lower tracings of A to G show that there was less than 1 msec shortening of neural conduction time from the weakest to the strongest stimulation.

In Fig. 44A-F the responses are evoked by graded sizes of parallel fibre volleys from a cell at a depth of 350_{μ} . The weakest stimulus (A) evoked a discharge with variable latency, and with strongest stimulation (B-E) there was shortening of latency and increase in the number of discharges; but even strong stimulation evoked only seven discharges (F). As in Fig. 43J, the identification

of this cell as an interneurone and not a Purkinje cell was confirmed by its responses to juxta-fastigial stimulation, where the latency of the spike was as long as 3.2 msec with weak stimulation and was reduced to 1.5 msec with strong stimulation.

The diminution of spike size during the height of the discharge is well shown in Fig. 44E and F. A remarkable feature in these responses was the apparent omission of a spike at the height of the discharge, which occurred with one of the four superimposed traces in E. and with all traces in F. After the omitted spike in the one trace of E the rhythm was resumed exactly in phase with the other three traces. There was a small deflection in E and F at the expected time of the rhythmic discharge; hence it can be postulated that there was generation of an impulse at the rhythmic firing centre of the cell, but its propagation was suppressed by the intense depolarization. This omission of a spike at the height of the rhythmic discharge has been observed with other interneurones in the cerebellar cortex and has previously been reported for Renshaw cells (Eccles, Eccles, Iggo and Lundberg, 1961).

G-K shows the responses of a much more superficially located cell (depth, 180_{μ}) to graded parallel fibre stimulation. Again, increase in size of the parallel fibre volley shortened the latency and increased the frequency and number of the discharges, there being a

curious partial spike at the strongest stimulation (K). The response (L) to juxta-fastigial stimulation with a latent period of 1.9 msec provided further evidence that this cell was not a Purkinje cell. The maximum frequency of response to a parallel fibre volley was no higher than 445 msec, which is a typical finding with such superficial cells, though it may also occur with some deeper interneurons (cf. Table 1).

The extremely powerful synaptic action of a parallel fibre volley on a deep interneurone (500_{μ}) is illustrated in Fig. 44N-Q. Differentiation from a Purkinje cell was aided by the juxta-fastigial response (M) with a latent period of 2.6 msec and in N-Q there was progressive increase of the parallel fibre stimulus from just above threshold (N) to three times threshold (Q). In the largest response (Q) the synaptic depolarization was so severe that there was disorganization of spike production and a fragmentation of spikes, which presumably is related to the process of spike suppression illustrated in Fig. 44E, F. This disorganization of spike generation was a common feature with the more powerful synaptic excitation of interneurons of the cerebellar cortex.

Double parallel fibre volleys

In Fig. 45 A to D, a strong conditioning stimulation of the parallel fibres was followed at various intervals by a second testing stim-

ulus applied through the same electrode. The control response to this testing stimulus is not shown. Facilitation at the briefest conditioning intervals (A, B) gives way to depression at longer intervals (C, D), the facilitation being largest at the briefest intervals (A). Three possible explanations may be offered for this facilitation: i) the conditioning stimulus may be followed by an increased excitability of parallel fibres that would result in an increase in size of the testing volley; ii) there may be a post-activation potentiation of the synapses of parallel fibres on the interneurone; iii) there may be facilitation of the responses to the testing volley by some general increase in excitability of the interneurone, for example by a residual depolarization.

A facilitation at brief testing intervals and depression at longer was exhibited by the superficially located interneurone (180_{μ} depth) in Fig. 45 E-M. For example, the control testing response of 4 spikes (F) was greatly increased at the shortest testing interval (30 msec in E), and with lengthening of the testing interval beyond 61 msec (H) this facilitation gave way to depression so that there was only a single spike at 150 msec (K), whereas at the longest interval (194 msec, M) there was recovery to 3 spikes.

Facilitation of the response to a second parallel fibre volley has been observed with every interneurone tested in the cerebellar cortex, and provides further evidence sharply separating them from Purkinje

cells. Since the conditioning volley produces a powerful and prolonged inhibitory depression of Purkinje cells, depression and not facilitation was always observed during the intervals in which maximum facilitation occurred in Fig. 45.

The time course of the facilitation and the subsequent depression has been displayed in Fig. 46 for the interneurone of Fig. 45 E-M, by plotting the number of spikes against the testing interval. Facilitation was maximal at the briefest testing intervals, and thereafter progressively declined to give place to depression with testing intervals in excess of 50 msec. It will be appreciated that the number of spikes in the testing response could not be properly assessed when the testing interval was so brief that it was superimposed on the conditioning response. However, under such conditions there were very high frequencies of spike discharge. No attempt has been made to determine the full duration of the prolonged depression, but it was in excess of 500 msec in Fig. 46.

It thus appears that with respect to the responses of the interneurons of the cerebellar cortex to the parallel fibre volleys there is no significant difference between the inhibitory interneurons which, by their depth in the cortex, might be classified as superficial stellate, basket and Golgi cells respectively. In order to subject the observed responses

to a closer scrutiny, there is in Table 1 an arrangement of the interneurons in order of depth below the cortical surface. Altogether in this present investigation 14 cells have been identified as interneurons, accurately located in depth and subjected to a sufficient investigation to justify their inclusion in the Table. In interpreting this Table it can be assumed that the respective depths of the somata for the three types of cells are: superficial stellate cells, 250_{μ} and superficial thereto; basket cells, 250 to 400_{μ} ; Golgi cells, 400_{μ} and deeper.

It is evident from Table 1 that the depth location of an interneuron in the cerebellar cortex is not associated with any characteristic features in its responses. This similarity in the properties of superficial stellate, basket and Golgi cells is not unexpected. From Golgi preparations it is known that the parallel fibre synapses are on dendritic spines, being in general similar to the spine synapses made by the parallel fibres on the Purkinje cells (Ramón y Cajal, 1911; Hátori and Szentágothai, 1964; Fox, Siegesmund and Dutta, 1964). The only difference so far noted by electronmicroscopy is that the dendritic spines of the basket cell dendrites are longer and more slender than the Purkinje cell spines (Hátori and Szentágothai, 1964), which agrees with the findings by Ramón y Cajal (1911). It will be shown that the excitatory synaptic action of parallel fibres on Purkinje dendrites has a duration in excess of 100 msec, which would

correlate with the long duration (both of the repetitive discharge 30 msec or more in Figs. 43, 44 and with the facilitation of a later testing response for at least 50 msec (Fig. 46). Presumably, because of the invaginated configuration of spine synapses, there is a relatively slow diffusion of the transmitter away from its site of liberation.

The frequency of the interneuronal discharge indicates that there is a relatively slow build-up in intensity of transmitter action. It seems likely that to a considerable extent this is due to the temporal dispersion of the synaptic bombardments by the individual parallel fibre impulses. There would be a fairly wide range of conduction times of the very slowly conducting impulses in these extremely slender parallel fibres.

C) DISCUSSION

a) Interpretation of the slow potential waves generated by a parallel fibre volley.

It will be simpler to consider first the responses of the chronically deafferented cerebellar cortex. Even in this preparation the applied surface stimulus may directly excite other structures besides the parallel fibres, namely the Purkinje cells and the other cells with

superficial dendrites - the stellate, basket and Golgi cells. However, the axonal branches of these cells have a very limited distribution in the longitudinal direction along the folium (Ramón y Cajal, 1911; Frezik, 1962; Szentágothai, 1965); hence potentials arising from their stimulation would be negligible when recording potential fields at 1 mm or more longitudinally from the site of stimulation, which was the usual situation in the present experiments. It is therefore justifiable to regard the potential fields as predominantly a consequence of the parallel fibre volley. An initial postulate is that the slow potential waves are produced not by the parallel fibre volley directly, but as a consequence of its synaptic actions, as specified below.

- (1) The parallel fibre volley would exert an excitatory synaptic action on the Purkinje cell dendrites by means of the crossing-over synapses on the dendritic spines (Gray, 1961; Fox, Siegesmund and Dutta, 1964; Hátori and Szentágothai, 1964). A subsidiary component of this same type would be the excitatory synaptic actions exerted on the neurones with dendrites in the molecular layer - the basket, stellate and Golgi cells (Eccles, Llinás and Sasaki, 1965c).
- (2) Inhibitory synaptic action on the bodies of the Purkinje cells is mediated by the parallel fibres basket cells pathway, cells which have an inhibitory synaptic action concentrated on the axonal poles of the soma of Purkinje cells (Szentágothai, 1963, 1964; Andersen,

Eccles and Voorhoeve, 1964; Hátori and Szentágothai, 1965).

(3) Inhibitory synaptic action on the dendrites of Purkinje cells, which is mediated by a path via superficial stellate cells, and is analogous to the basket cell pathway. (4) Inhibitory synaptic action on the dendrites of the granule cell that also has a similar path, the inhibitory cells being the Golgi cells (Eccles, Llinás and Sasaki, 1964). Since these inhibitory synapses are in the granule layer at a depth in excess of 400_{μ} , the potential fields would only contribute effectively to the deepest potentials and so their contribution will be mentioned only incidentally in the present discussion.

The long time course - in excess of 100 msec - of the synaptic excitatory action directly exerted by a parallel fibre volley on the Purkinje cell dendrites has been demonstrated by the facilitation of the antidromic invasion of those dendrites which lie within or close to the excited beam of fibres (Eccles, Llinás and Sasaki, 1965b). When a relatively weak stimulation is applied through a LOC. electrode, as in the present experiments, the excited beam of parallel fibres is concentrated in the superficial zone of the molecular layer. There is always a rapid decrease of the spike potential of the parallel fibre volley at depths below 100_{μ} (cf. Figs. 36, track A; 40A), and it may even be undetectable at that depth (Figs. 32C, 33D). As a consequence of this superficial location, the activated ex-

citatory synapses will be concentrated on the most superficial dendritic branches, which become sinks for extracellular current flow from the passive sources on the deeper dendrites. This current flow provides a satisfactory explanation for the potential profiles recorded in tracks through the excited beam of parallel fibres (Figs. 37A, tracks A, D; 38), a positivity under the excited beam of parallel fibres steeply grading to the relative negativity at the superficial level of the beam.

Tests utilizing the antidromic invasion of the Purkinje cell soma and dendrites provide a means of demonstrating that, in addition to its superficial excitatory action, a parallel fibre volley results in a prolonged inhibitory action - also in excess of 100 msec - applied not only at the somata, but also at the dendritic level of the Purkinje cells. A conditioning parallel fibre volley considerably depressed the antidromic spike potential extracellularly recorded at the level of the Purkinje cell somata; but there was an even larger depression more superficially, at the dendritic region, the maximum being attained at a depth 250 to 200 μ , (see Chapter 7), which is approximately midway between the somata origin and the superficial termination of the dendrites. Evidently there is a very effective synaptic inhibitory action on the dendrites as well as the basket cell inhibition on the soma of a Purkinje cell. In line with anatomical

evidence it can be postulated that this dendritic inhibition is effected by the superficial stellate cells, which are the homologues (Ramón y Cajal, 1911) of the basket cells. The inhibitory synapses on the dendrites and somata of the Purkinje cells would cause these regions to be sources of current to passive sinks on the superficial dendritic branches and on the axon collaterals. Hence an explanation is provided for a potential profile (cf. Fig. 37A at 400 μ laterality) with a maximum positivity at 200 to 400 μ depth and a lesser positivity (or a relative negativity) superficial and deep thereto.

The conditions obtained with a schematic Purkinje cell having a superficial dendritic excitation in response to a LOC. stimulation are shown in Fig. 42A with the convention adopted in the treatment of potential profiles produced by antidromic invasion (Eccles, Llinás and Sasaki, 1965b). Since all the Purkinje cells in an area will be subjected to a similar synaptic excitation, as a first approximation the extracellular current flow for each can be regarded as confined to a narrow cylinder private to that cell. The potential profile (B) generated by that current is shown with the steepness of gradient matching the current density, the potential reversing from a superficial negativity to attain the maximum positivity considerably deeper than the excited dendritic zone. Deeper than this maximum, the positivity is shown to decline slowly, an effect which would be

expected due to the wider distribution and the less intense current flow that would arise because of the small area of the uniform dendritic excitation relative to these larger vertical depths. These extracellular currents would as a consequence loop widely, as with the standard dipole in volume. A further complicating factor is the wide transverse spread, at least 350_{μ} (Fox and Barnard, 1957) of dendrites of a Purkinje cell. However, the transverse components of the many overlapping dendritic trees would approximately cancel, leaving the dominant vertical components illustrated in A.

With tracks progressively more lateral to the centre of the excited beam of parallel fibres, there would be a rapid decrease in the excitatory synaptic activity on the dendrites and consequently a rapid decrease in the steepness of the profile. Fig. 42C shows with the same convention the extracellular current flow for a uniform distribution of active inhibitory synapses over the soma and the dendrites up to 150_{μ} from the surface. The potential profile (D) so generated is seen to be a relatively gentle slope from the surface to a maximum positivity that would be maintained from about 250 to 400_{μ} depth, while still deeper there would be a gentle decline. Since the basket and stellate cells have axonal distributions running transversely from their dendrite-soma location in and beneath the excited beam of parallel fibres Fig. 32A, this potential profile generated by inhibitory synapses would be expected in this lateral position, and this is in fact

observed with the track C in Fig. 37A at a laterality of 400_{μ} . On the other hand the potential profile for the track right on beam should be dominated by the excitatory synaptic activity, since in Fig. 42A, B, and in Fig. 37A, track A, this profile had the expected features of a very steep potential gradient to a depth of 100_{μ} , there being a maximum positivity at 150_{μ} depth and deeper thereto a gentle decline.

This theoretical treatment neglects many complicating factors, and hence can provide merely a first approximation in the attempt to explain the production of the slow potential waves. However, it does give some justification for attempting to dissect the observed wave forms into their constituents. When attempting this exercise it is reasonable to assume that there are similar time courses for the currents produced by excitatory and inhibitory synapses, because their respective excitatory and inhibitory actions on the invasion of the Purkinje dendrites by antidromic impulses have similar time courses (Eccles, Llínás and Sasaki, 1965b). The size and time course of the potential wave produced by the parallel fibre alone, uncomplicated by synaptically induced potentials, is illustrated in Fig. 40E, where the cerebellar cortex was anoxic due to circulatory failure. As often observed in such cases the spike potential of the parallel fibres survived for many minutes after the failure of all other potentials.

Fig. 42E gives a diagrammatic illustration of the manner in which the potential observed on beam and at the surface is compounded of a triphasic spike potential with a time course derived from Fig. 40E, and a large slow negative wave that begins during the early negativity. The evidence for this early origin will be given later. The contribution of the inhibitory synapses to the late negativity would be negligible, because they are largely distributed some hundred of microns lateral to the beam (Szentágothai, 1963, 1964, 1965). At 100_{μ} depth (E) the triphasic spike is much smaller (cf. Fig. 40E) and the excitatory synapses now produce a large positive wave as shown in the potential profile (Fig. 42B). Again the inhibitory synapses may have a relatively small action. By contrast at 400_{μ} off-beam no detectable potential would be produced by the parallel fibre impulses and their excitatory synaptic action, and the inhibitory synapses would also be negligible at the surface and small at 100_{μ} depth, but would give a large positive potential at depths from 200_{μ} to 400_{μ} (G). More complex problems of dissection are of course involved at intermediate degrees of laterality, but the same general principles apply.

In some experiments there has been an indication of a relatively large extension of the positive wave down into the granular layer. This may be seen in the contour plot of Fig. 38A at a depth of 600_{μ} and at about 200_{μ} lateral to the centre of the beam of excited paral-

lel fibres. It would be expected that the Golgi cells excited by the parallel fibres would produce a positive potential at this depth as a consequence of their postsynaptic inhibitory action on the granule cells (Eccles, Llinás and Sasaki, 1964b).

a) Interpretation of slow potential waves induced by a parallel fibre volley conditioned by a preceding volley.

The above suggested modes of production of the potential fields by a parallel fibre volley have been shown to be in accord with the experimental evidence provided by the responses to single parallel fibre volleys. They also allow the development of explanations of the diverse results on the conditioning of slow negative and positive potentials by a preceding parallel fibre volley.

(1) The potentiation of the slow negative wave (Figs. 34, 35, 39, 40B) would be a further example of the potentiated synaptic action produced by repetitive stimulation (Curtis and Eccles, 1960; Eccles, Hubbard and Oscarsson, 1961; Hubbard, 1963; Eccles, 1964, pp. 83-90). The effectiveness of this potentiation is well displayed by the increased repetitive discharge which a second parallel fibre volley evokes from basket, superficial stellate and Golgi cells. In the case of Purkinje cells the potentiation of the synaptic excitatory action is not displayed by an increased discharge because this is prevented by the powerful inhibitory action exerted by the basket and superficial stellate cells (Figs. 41; Andersen, Eccles & Voorhoeve,

1964; Eccles, Llinás and Sasaki, 1965b, 1965e).

(2) It has been postulated Fig. 42F, G, that the slow positive wave recorded deep to the beam of excited parallel fibres, F, and also at all levels lateral to this beam is partially attributable to the source for the sinks on the synaptically excited dendrites of the Purkinje and other cells, as well as to the inhibitory action exerted by the superficial stellate and basket cells on the dendrites and somata of the Purkinje cells respectively. The diverse effects of conditioning on the size of the positive wave (Figs. 39J-O, 40A-E) can then be ascribed to various antagonistic effects; for example, to sink-source antagonism within the fields produced by the excitatory synapses as in Fig. 40B at 70 and 100 μ depth; and also to possible antagonistic effects operating on the inhibitory synaptic action during the conditioning - the increase in the number of impulses discharged by the inhibitory cells, and to a possible decrease in the effectiveness of the inhibitory synapses during repetitive bombardment (Eccles, Llinás and Sasaki, 1965e). Finally, the positive wave produced as a consequence of direct mossy fibre stimulation (Fig. 33C, D) is greatly depressed by the action of the conditioning parallel fibre volley in inducing inhibition of the mossy fibre granule cell synapses through action of Golgi cells (Eccles, Llinás and Sasaki, 1964b and Figs. 27, 28 and 31 of this thesis). For example, a considerable part of positive wave in Fig. 40C is produced as a consequence of mossy fibre stim-

ulation by the LOC. stimulus, and the large inhibition of the positive wave produced by the second LOC. stimulus in Fig. 40D is attributable to the Golgi cell inhibition. The effect of conditioning by a preceding LOC. stimulation on the intracellularly recorded potentials was shown in Fig. 31.

In Fig. 40B at depths of 0 to 70_u the field potential of the parallel fibre volley evoked by the second LOC. stimulus showed a remarkable difference from the corresponding control records in Fig. 40A. The initial positive component of the spike was reduced in size, though the total height of the fast potential (at 50 and 70_u) was not reduced. Evidently this increase in the slow negativity which is very apparent after the fast negative component of the parallel fibre field is superimposed on the preceding slow negativity, lifts up the summit of the fast potential field and begins before the initial positive component attains its maximum.

This effect is best measured by expressing the initial positive component as a percentage of the total diphasic fast potential and comparing this with the percentages for the conditioned and control responses. For example, in Fig. 40A, B, the respective percentages were 13 and 20 at 50_u depth, and 16 and 21 at 75_u ; in Fig. 34 at 9.2 msec stimulus interval the conditioned and control responses were 13 and 23%; in Fig. 35, at 11.2 msec, 13 and 31%; and, in Fig. 39 D

and E, 15 and 16%, as against 24 in the control. In all these examples there was a considerable potentiation of the slow negative wave of the conditioned response. By contrast, when there was little or no potentiation of the slow negative wave after the second parallel fibre spike, there was a negligible conditioning effect. For example, in Fig. 40C at depths of 25 and 50_u the same measurement for the conditioned response gave 28 and 29% respectively as against 30% for each control.

There is thus a uniformly good correlation between the diminution of the initial positive spike and the size of the slow negative wave after the spike. On this account it is postulated that the slow negative wave actually begins before the summit of the initial positive spike, and it was so plotted in Fig. 42E. It is difficult to account for such an early origin for a negative wave that has been identified with the EPSP produced by the parallel fibre volley. It seems necessary to postulate that some parallel fibre impulses travel faster with the consequence that the main volley finds some EPSP already initiated by the time of its arrival.

The inhibitory action of Purkinje axon collaterals will be demonstrated for Purkinje cells in the deafferented cerebellum (Chapter 7). These same axon collaterals also inhibit basket and Golgi cells (Eccles, Llinás and Sasaki, 1955c), and thus, would by disinhibition (Wilson and Burgess, 1962), serve as a recurrent facilitation mechanism for the Purkinje cell. (See Fig. 63).

VI A) THE ORGANIZATION OF THE PURKINJE CELL INPUTS ACTIVATED BY THE M.G.R.

The preceding chapters have described how the mossy fibres come eventually to act both in an excitatory and inhibitory manner on the Purkinje cells. The investigations described there utilized extracellular recording with microelectrodes to give two kinds of information: the extracellular potentials and their synthesis in the plotting of the laminated potential fields developed in the cerebellar cortex by specific methods of electrical stimulation; and the extracellularly recorded responses which these same stimulations evoke in the various types of cells in the cerebellar cortex.

In the present chapter, intracellular recording from Purkinje cells will be used to illustrate three points: the mode of action of the various excitatory and inhibitory synapses on Purkinje cells, the reactions of Purkinje cells to these synaptic actions and the generation of impulses by Purkinje cells.

a) Orthodromic activation of Purkinje cells recorded intracellularly.

It has been described previously, that a stimulus applied through a concentric electrode on the surface of a cerebellar folium (LOC.) excites a volley of impulses in the parallel fibres which in turn activate Purkinje cells by means of the crossing over synapses. An intracellular record from a Purkinje cell during a LOC. stimulation is il-

illustrated in the lower traces of Fig. 47A, B. This stimulation evoked a spike potential with a latency of 3.5 msec and a voltage of about 65 mV. In the more highly amplified upper traces the spikes are seen to arise from slowly developing depolarizations with onsets approximately indicated by the arrows. In these records there is a slowly rising background depolarization, which distorts the potentials subsequent to the spike potentials, nevertheless, it would appear in A and B that the spike potentials were followed by prolonged hyperpolarizations.

In C and D stimulation was applied by a concentric needle electrode which had been inserted into the neighborhood of the fastigial nucleus (Granit and Phillips, 1956), the J.F. electrode. The J.F. stimuli failed to excite the axon of the impaled Purkinje cell, but evoked a later complex of spike potentials, an initial spike rising from a slowly developing depolarization, and about 1.5 msec later the spike potential complex that has been shown to be produced by the climbing fibre innervating that Purkinje cell (Eccles, Llinás and Sasaki, 1964a). The earlier slowly rising depolarization with the single superimposed spike must be produced as a consequence of the excitation of the mossy fibres, for these are the only fibres, other than the climbing fibres, which pass from the region of the J.F. electrode to the cerebellar cortex, where they excite the discharge of impulses from granule cells along their axons, the parallel fibres.

Thus the initial responses in C and D were produced by the synaptic excitatory action of parallel fibre impulses, in the same manner as the responses in A and B. There is a general similarity of these responses, the only difference being that in the J.F.-evoked responses the spike arose from a much larger synaptic depolarization than in those evoked by the LOC. stimulation.

Fig. 47E, F were recorded from a Purkinje cell immediately after a particularly good impalement, the resting membrane potential being as high as -60 mV and the spike potentials 98 mV, the largest ever observed. The J.F.-evoked spike (F) arose from a small slowly developing depolarization resembling that in C and D, but rather smaller. By contrast in E there was only a trace of a slow depolarization preceding the LOC. evoked spike. Both spike potentials were followed by a prolonged hyperpolarization. Evidently, the J.F. stimulus excited neither the axon nor the climbing fibre of the impaled Purkinje cell, the whole potential complex of F being attributable to mossy fibres.

Record G gives an example of the sequences of potentials when the J.F. stimulus excited the axon of the Purkinje cell in addition to mossy fibres. Following the initial antidromic spike potential, 59 mV with a latency of 0.6 msec there was a brief hyperpolarization which was terminated by a depolarization leading on to a spike potential having a total latency of 3 msec. In this case there was also

a later prolonged hyperpolarization.

The series of Fig. 47H, I, J, is interesting because in H and I the depolarization produced by the J.F. stimulus did not initiate a spike potential as it did in J (56 mV), and in all other records displayed in Fig. 47. It appears in H and I that the initial depolarization passed over to a hyperpolarization, just as it did after the spike potential in all other records of this figure, except C and D where there was the superimposed climbing fibre response.

The differing distribution of the excitatory synapses on the Purkinje cell dendrites (Fox, Siegesmund and Dutta, 1964) accounts satisfactorily for the finding that the spike arises from a larger EPSP with J.F. stimulation (C, D, F) than with LOC. stimulation (A, B, E). Presumably with J.F. stimulation there will be excitation of parallel fibres at all depths of the molecular layer, whereas with the LOC. stimulation there would only be a superficial band of excited parallel fibres. In this latter case the excitatory synaptic action would thus be on the superficially located Purkinje dendrites, and hence on the average more remote from the recording electrode in the soma. This explanation must assume also that with the LOC. stimulation the impulse initiation occurred in the superficial dendrites, whereas the J.F.-evoked impulse, arose much closer to the recording site in the soma. This assumption is justified since as will be shown later, antidromic impulses propagate up the den-

drites at least 200_{μ} beyond the soma and that this propagation is extended even further by synaptic excitatory action on the dendrites.

b) Excitatory and inhibitory postsynaptic potentials of Purkinje cells

In Fig. 48A-F are the responses of a Purkinje cell to a progressively increasing LOC. stimulus, the intensities being indicated in arbitrary units. The field potential evoked by the strongest stimulus was recorded after withdrawal of the microelectrode to a just extracellular position and is shown in F immediately below the corresponding intracellular response. Evidently there was an initial membrane depolarization (an EPSP) of 3.6 mV followed by a hyperpolarization (an IPSP) which developed to attain a maximum of 3.0 mV towards the end of the trace. With the stimulus strengths of 60, 70 and 80 there was also a brief initial EPSP preceding the IPSP, and presumably there was also an initial EPSP in A and B; but, in the absence of extracellular recordings at these stimulus strengths, the small initial upward deflections cannot be certainly identified as EPSPs.

The latent periods of the EPSPs in C to F were about 3.2 msec, while presumably the latencies of the IPSPs can be measured at the points of the sudden downward deflections which terminated the summits of the EPSPs. Measured in this manner the IPSP latencies shorten-

ed from 4.5 msec in C to 4.2 msec in E. and F. The summits of the hyperpolarizations were attained progressively later in B to F. However, this need not indicate progressively later summits for the IPSPs, because alternatively it could be due to the larger EPSPs being superimposed in records C to F.

It should be noted that increase in the strength of stimulation from 60 to 100 (C to F) increased the IPSP by only 10%, whereas the EPSP was many times larger. This differential would be expected because the increased LOC. stimulus would excite deeper parallel fibres and hence synapses closer to the recording electrode, whereas, with all strengths of LOC. stimulation, the IPSP would be produced by the inhibitory synapses of the basket cells and stellate cells, there being no progressive advantage from closer proximity. It can moreover be envisaged that, above a certain level, increased excitation of basket and stellate cells does not give a commensurate increase in the IPSP, there being a type of occlusion due to the approximation of the IPSP to the inhibitory equilibrium potential.

A more usual series of intracellular responses to graded LOC. stimulation is illustrated in Fig. 48G-K together with the corresponding extracellular field potentials. Subtraction of these extracellular potentials shows that there was no detectable phase of initial membrane depolarization as in C-F, the hyperpolarization having a latency of about 6 msec. Nevertheless, the series of L to P reveals that there

was a large EPSP which began with a latency of about 5 msec. By applying a steady hyperpolarizing current through the recording microelectrode, the IPSP was decreased and the EPSP increased with the consequence that the EPSP-IPSP balance normally obtained (L) was biased in M to P progressively further in favor of the EPSP. In G-K progressive increase in the IPSP was not associated with a slowing in its time course.

The unusually large initial EPSP in Fig. 48D-F may be correlated with the very superficial location of the intracellular electrode—about 200_{μ} from the surface. At such a depth the electrode must have been inserted into one of the large dendrites and hence was much more favourably placed for recording the EPSPs evoked by the parallel fibre volley.

In Figs. 49 and 50 A-H are assembled records from the most stable and prolonged intracellular investigation on a Purkinje cell. The recorded membrane potential continued to be about -50mV throughout almost an hour of investigation. Perhaps the stability of this penetration is attributable to the depth (800_{μ}), which indicates that the microelectrode entered the Purkinje cell during a tangential traject as the cortex curved from the surface of folium down into a sulcus. Because of this excessive depth it would be expected that the LOC. stimulus would evoke virtually no EPSP; but on the other hand it would be quite effective in evoking an IPSP because of the

transverse distribution of the basket cells (cf. Szentagothai, 1963, 1965; Andersen, Eccles and Voorhoeve, 1964; Hamori and Szentagothai, 1965).

Fig. 49 D shows at high and low amplification both the intracellular and extracellular potentials evoked by a LOC. stimulus. Subtraction of the latter from the former shows that the induced membrane potential change was solely a hyperpolarization (IPSP) having a latency of 3.8 msec and a voltage of -22mV . A weak J.F. stimulus (A) also evoked an IPSP of similar time course but smaller size. Stronger J.F. stimulation (B, C) excited the climbing fibre supplying this Purkinje cell, and, following the depolarization so induced, there was the prolonged hyperpolarization characteristic of the IPSP. Subtraction of the corresponding extracellular potential in C gives a value of -14 mV for the IPSP.

The same courses of the IPSPs produced by J.F. and LOC. stimulation can be compared in the respective series, F-I and J-N, which were recorded at a slower sweep speed. Both of these series were recorded at high and low amplifications, the potentials being evoked by graded stimulations at the indicated strengths scaled in arbitrary units. The J.F. stimulation in H and I evoked an initial climbing fibre response, just as in B and C. However, subsequently to this response, the IPSP had virtually the same time course as when it was uncomplicated in this way, either with weaker J.F. stimulation (F-G)

or with LOC.-evoked IPSPs of comparable size (J-N). In O to S are a further series of J.F.-evoked responses with stimulus strengths on the same scale as in F to I, but at a still slower sweep speed. A total duration of 160 msec for the IPSP is indicated by these records, which is in very good agreement with the duration of the inhibitory action on the antidromic invasion of Purkinje cells (Fig. 59).

Throughout the series E to S small spike-like potentials appeared at a fairly regular frequency of almost 100 msec. Similar potentials were observed by Granit and Phillips (1956), the rhythmic wavelets or prepotentials. Possibly these potentials are generated by a rhythmic firing locus on some injured area of the cell, for example a small dendrite damage by the micro-electrode. However this may be, the response in Fig. 49 was valuable in signalling the duration of the IPSP, because it was completely suppressed throughout almost the whole duration of the IPSP; and, when it reappeared at the terminal stage of the IPSP, it initially fired at a low frequency and it only resumed its initial frequency after the termination of the IPSP. It was thus a sensitive indicator of low levels of inhibitory action.

Besides the IPSPs produced by LOC. and J.F. stimulation, there are in this figure several examples of spontaneously evoked IPSPs, which are indicated by arrows in E, F, J, K, M, P, and S. During these IPSPs the spontaneous spikes were usually suppressed, though

with the smaller IPSPs there may be merely a slowing of frequency. This criterion of slowed frequency suggests that there are several other very small spontaneous IPSPs in records O-S. There are also four examples of large spontaneous depolarizing potentials in K, Q and S (two). These are the climbing fibre responses which have already been reported as occurring spontaneously.

c) Effect of applied transmembrane currents on inhibitory postsynaptic potentials

Fig. 50C gives a control record of the IPSP (-15mV) produced by a Loc. stimulus in the same Purkinje cell as in Fig. 49. In A the IPSP was increased to -28 mV by a strong depolarizing current, and in B to -21 mV by a weaker current. Series D to H show the effect of progressively increased hyperpolarizing currents, firstly to decrease the IPSP and then in H to reverse it to a small depolarizing response. In G there was apparently a diphasic positive-negative potential. It seems unlikely that in a Purkinje cell at depth 800_{μ} there would be an appreciable synaptic excitatory action to give responses similar to those of Fig. 48 M-P. Comparable positive-negative phases have been observed for pure IPSPs of motoneurons subjected to a hyperpolarizing current insufficient for their complete inversion (Coombs, Eccles and Fatt, 1955a; Llinas and Terzuolo, 1965. Presumably the same explanation suffices, namely, the non-uniform change which

the applied current produces in the subsynaptic membranes of the various inhibitory synapses. Those close to the microelectrode suffer a potential change beyond the reversal potential for the inhibitory synaptic currents, while with the more remote synapses the potential change was inadequate for reversal.

In the series of Fig. 51 the intracellular microelectrode was filled with 3M KCl, and, the diffusion of Cl^- ions had increased the Cl^- ion concentration in the impaled cell so that the IPSP evoked by a J.F. stimulation was inverted to a depolarizing potential (Trace M), just as occurs with the IPSP of motoneurones (Coombs, Eccles and Fatt, 1955a) and many other types of neurones. In O-T the control depolarizing IPSP (O) diminished (P) and then reversed (R-T) to a progressively larger hyperpolarizing IPSP by increasing strengths of depolarizing DC currents. An additional feature of interest is provided by the small depolarizing potential (indicated by arrows) sometimes superimposed at a latency of 8 msec, and that was identified as a climbing fibre EPSP. In contrast to the IPSP this EPSP remained as a depolarizing potential, though a little reduced, even during the largest depolarizing current. It thus demonstrates that there is a differential effect of applied DC current on EPSPs and IPSPs, even when increased intracellular chloride has inverted the latter to depolarizing potentials (cf. Coombs, Eccles and Fatt, 1965a, b).

d) The inhibitory synaptic noise of Purkinje cells

In Fig. 49 there were numerous examples of spontaneously occurring IPSPs that appeared to be identical with small evoked IPSPs, as for example at arrows in E, F, J, K, M, P and S. In Fig. 48I and J the small spontaneous hyperpolarizations are presumably also IPSPs. Granit and Phillips (1956, Fig. 7) illustrated two similar examples of spontaneous IPSPs, the small hyperpolarization silencing the repetitively discharging Purkinje cell for about 100 msec. The inhibitory interneurons of the cerebellar cortex uniformly display spontaneous discharges at a low (usually between 7 and 30 msec) and rather irregular frequency (Eccles, Llinás and Sasaki, 1965c); hence these small spontaneously occurring IPSPs can be attributed to these discharges; and these IPSPs may appropriately be termed inhibitory synaptic noise, being the counterpart of the excitatory synaptic noise first described by Brock, Coombs and Eccles (1952). It is thus postulated that single impulses in the presynaptic terminals of a basket cell are responsible for producing these randomly occurring IPSPs of Purkinje cells in Figs. 48 and 49. The inhibitory synaptic noise has a magnitude in accord with this postulate. As many as 30 and up to 50 basket cells make synaptic contact with one Purkinje cell (Ramón y Cajal, 1911; Fox 1962; Szentágothai, 1965), but some of these contacts are much more extensive than others; hence the expectation

that the inhibitory noise would display the observed range of size when measured, relative to the maximum evoked IPSP in the records.

Particularly favourable conditions are provided for recording the IPSPs on inhibitory noise when they have been inverted by a large increase in intracellular concentration of chloride ions as in Fig. 51. It has already been pointed out that the action of applied depolarizing currents in Fig. 51, O-T sharply distinguished between the depolarizing IPSPs and EPSPs for the Purkinje cell giving the responses of Fig. 51 A-N. In the three superimposed traces (A) there are nine randomly occurring unitary IPSPs having a size range from 0.5 to 4.3 mV. LOC. stimulation of progressively increasing strength (B-G) evoked IPSPs of similar time course and as large as 10.3 mV. The smallest evoked potentials in B and C had a size comparable with the inhibitory noise. LOC. stimulation of strength 50 or more suppressed all inhibitory noise for the duration of the trace, and in records H to N this suppression also occurred even with the weakest J.F. stimulation (H). The IPSPs of the two traces in H are within the size range for unitary responses, but alternatively the larger could be a double response. The superimposed traces in D and I give a clear indication that the evoked IPSPs were compounded of varying numbers of unitary IPSPs.

- e) The action of LOC. stimulation in conditioning the intracellular responses evoked by LOC. or mossy fibre stimulation.

The interaction of two LOC. stimuli at various intervals was reported by Andersen, Eccles and Voorhoeve (1964, Fig. 3B). At long stimulus intervals a large additional IPSP was produced, but with shorter intervals the additional IPSP was depressed so that the summed IPSP resulted in a hyperpolarization no larger than that produced by the first alone, i.e., there appeared to be an occlusion of the IPSP. Correspondingly, there was a large uncovering of the EPSP produced by the LOC. stimulation, just as was effected by a background hyperpolarization in Fig. 48 L to P.

In Fig. 52A-F the second LOC. stimulus was a little stronger than the first, as is shown by the larger IPSP in the control response (F). There was a considerable depression (50-65%) of the added IPSP even at the long intervals (D, E) and at the briefest interval (A) the second IPSP did not reach a more hyperpolarized level than the control response in F. The series G-M was produced by stimulation through two different LOC. electrodes in close proximity, but the control IPSP evoked by the second (M) was smaller than the first. There was a severe depression of the IPSP added at the long intervals of K and L, and at briefer intervals (H-J) an almost complete suppression uncovered a small EPSP, while at the briefest interval (G) there was only a small

additional IPSP during the declining phase.

Intracellular recording from the chronically deafferented cerebellum in Fig. 53 shows in E-L an interaction of the responses to two LOC. stimuli very like those of the normal cerebella in Fig. 52. There was occlusion of the IPSPs at brief intervals and a consequential uncovering of the EPSPs in E-I. The series at slow sweep (I-L) shows the eventual complete recovery of the IPSP response (K, L). The series with graded LOC. stimulation (A-D) resembles that of Fig. 48 G-K in a normal cerebellum.

B) DISCUSSION

a) Inhibitory synaptic noise

The inhibitory synaptic noise displayed in Figs. 48 and 49 does not seem to be quantal like the miniature endplate potentials, but rather, to be attributable entirely to the unitary synaptic action of basket cell impulses, since it was completely suppressed for at least 100 msec after a LOC. or J. F. stimulation (Fig. 51 C-N). Such stimulation inhibits the discharge of basket cells (Eccles, Llinás and

Sasaki, 1965c) probably by the inhibitory action of Golgi cells on the mossy fibre-granule cell background excitatory path to basket cells. The wide range in size of the spontaneous unitary potentials in Fig. 49 may be due to variations in the quantal composition of the unitary IPSPs, but alternatively the smaller IPSPs could be produced by inhibitory synapses remote from the recording site. In attempting to correlate the mean frequency of the inhibitory noise with the frequencies observed for the spontaneous background discharges of basket cells (7 to 30 msec), allowance must be made for the innervation of each Purkinje cell by up to 50 basket cells (Szentágothai, 1965).

b) The EPSP and IPSPs generated by a parallel fibre volley

When a LOC. stimulation has generated both an EPSP and an IPSP of a Purkinje cell, the latency of the former has always been briefer - usually by 1 to 2 msec, as in Figs. 48, 52, 53. The present observations are in accord with the previous findings of Andersen, Eccles and Voorhoeve (1964) and with their postulate that this differential is attributable to an interneurone in the inhibitory pathway. As illustrated in Fig. 2, the EPSP would be produced by the direct excitatory action of the parallel fibre impulses at the crossing-over synapses on the dendritic tree of the Purkinje cells (Fox, Siegesmund and Dutta, 1964; Hátori and Szentágothai, 1964), whereas the inhibitory pathway for parallel fibre impulses would lead through either basket cells or

superficial stellate cells (Ramón y Cajal, 1911; Jakob, 1928; Fox, 1962; Hátori and Szentágothai, 1964, 1965) and therefore to the inhibitory synapses on Purkinje cells.

As mentioned above, the location of the intracellular microelectrode makes a biased record of IPSPs generated by the basket cells with their synapses concentrated on the axonal pole of the soma. Evidence for the mediation of superficial stellate cells in the inhibitory action of parallel fibre volleys has been provided solely by the extracellular recording both of the potential profiles in the molecular layer and of the inhibitory action on antidromic propagation along the Purkinje cell dendrites (see next chapter). There is good correlation between the distribution of this inhibitory action along the dendrites, with a maximum at 200 to 300 μ depth, and the potential profile produced some hundreds of microns lateral to a parallel fibre volley, which usually exhibited a maximum positivity at this depth and which indicates that this is the location for the sources induced by activated inhibitory synapses. There is histological evidence that the axonal terminals of the superficial stellate cells are most concentrated at such a depth and that there is no alternative pathway whereby parallel fibre impulses could activate inhibitory synapses on Purkinje cell dendrites (Ramón y Cajal, 1911; Jakob, 1928; Szentágothai, 1964). By electrotonic spread, inhibitory synaptic action on the dendrites would contribute to the IPSP recorded in a Purkinje cell soma, but it is not

possible to distinguish this subsidiary component from that produced by the much more favourably located inhibitory synapses on the soma. However, the large unitary IPSPs of synaptic noise (Figs. 48 and 49) could be produced only by the synapses on the soma, presumably of the basket cells.

Though intracellular recording from Purkinje cells, particularly in the large dendrites (Fig. 48 A-F), has demonstrated the production of EPSPs by the action of parallel fibre volleys, it has not provided satisfactory evidence on the time course of these EPSPs. The parallel fibre volley also always evokes an IPSP which rapidly reverses the initial depolarization of the EPSP. The time course of the EPSP could therefore be determined only if the IPSP were neutralized by application of a steady hyperpolarizing DC current of suitable strength. Fig. 48 M-P gives an example of how this technique uncovers an EPSP having a duration of at least 20 msec, but a reliable determination of time course could require a much more carefully controlled experiment with of course in addition, the superimposition of control curves for the applied current.

A stimulus applied through a LOC. electrode excites a fairly narrow beam of parallel fibres and has an excitatory synaptic action on those Purkinje cells with dendrites ramifying in this band. Because of the extensive transverse spread of the dendrites of a Purkinje cell,

the zone of excited cells will be considerably wider than the band of parallel fibres; nevertheless the transverse spread of the inhibitory synaptic action exerted by basket and stellate cells with their transversely directed axons should be much more extensive - at least 500_u from each side of the excited beam of parallel fibres (Szentágothai, 1963, 1964, 1965). The contour diagrams of extracellular field potentials show this expected spread of the positive field potential (Fig. 37 and 38). No systematic attempt has been made to sample the EPSPs and IPSPs of Purkinje cells at varying transverse displacements from the excited beam of parallel fibres, but our random sampling shows that hundreds of microns "off-beam", the IPSPs are still large (cf. Fig. 50), whereas EPSPs could not be detected.

In the interpretation of the extracellular potential fields set up by LOC. stimulation, it has been postulated that a considerable part of the positive potential wave recorded in the depth of the molecular layer and even deeper, is generated by the inhibitory synaptic action of superficial stellate and basket cells. It has been argued that the latency of inhibitory action in Purkinje cells is not inconsistent with this interpretation of the extracellular positive wave (Eccles, Llinás and Sasaki, 1965b,d). Nevertheless it may appear that the duration of this positive wave (10 to 20 msec) is far too short for it to be the extracellular counterpart of the IPSP, which has a relatively slow increase

to a maximum at 10 to 15 msec and a total duration usually in excess of 100 msec. However, it is the flow of currents generated by the inhibition synaptic action that produces the extracellular positivity; and, as with other types of synaptic action (Eccles, 1964, pp.152-155), it would be expected that the inhibitory current would be large only during the rapid rise of the IPSP and would tail off to a negligible level during its declining phase, which to a considerable extent would be dependent on the electric time constant of the membrane. The residual flow of inhibitory synaptic current during the declining phase of the IPSP could well be so small that there would be no appreciable extracellular field, as has been argued for similar observations with hippocampal pyramidal cells (Andersen, Eccles and Løving, 1964). Likewise with Purkinje cells there may be no inconsistency between the durations of the IPSPs and of the extracellular positivities.

The depression of antidromic propagation into Purkinje cells has been employed to map the time course of the inhibitory action exerted as a consequence of a LOC. stimulus (Fig. 59 and 60). The time course so determined by the extracellular responses of Purkinje cells revealed a maximum inhibition at test intervals of 20 to 40 msec and a total duration of about 140 msec, which is in good agreement with the intracellularly recorded IPSP. It can be concluded that in normal Purkinje cells the inhibitory action evoked by the stimulation is prod-

uced for the most part by the hyperpolarization of the IPSP and that this inhibitory hyperpolarization is not an artefact arising because of the depolarized state of the Purkinje cells impaled by microelectrodes. The extracellular positive waves give further evidence that inhibitory synaptic action induces a considerable hyperpolarization of Purkinje cells with resting potentials undisturbed by an impaling microelectrode.

c) Production of IPSPs by a parallel fibre volley when conditioned by a preceding parallel volley.

Complex problems are involved in attempting to account for the varied degrees of depression exerted by a conditioning LOC. stimulus on the IPSP produced by a later testing stimulus. In Fig. 52 G-L there was a very severe depression, which was almost total at the shorter stimulus intervals of G-J, whereas there was much less depression in another Purkinje cell from the same experiment (A-F). Yet it has been shown that under such conditions there was always a considerable potentiation of the basket cell discharge evoked by the second volley. Three explanations may be given for the depression of the IPSPs despite potentiation of basket cell responses: depletion of transmitter in the synaptic terminals of the basket cells; desensitization of the receptor for the inhibitory transmitter; approximation of the membrane potential of the conditioning IPSP to the equilibrium potential for the inhibitory

synaptic currents of the test response. Finally, the testing LOC. stimulus can produce some of its IPSP by exciting basket cells by the pathway: mossy fibres to granule cells to parallel fibres to basket cells. The conditioning LOC. stimulus would depress the IPSPs so produced by the path - parallel fibres to Golgi cells, which are inhibitors of the mossy fibre-granule cell synapses.

There appears to be a serious inconsistency between the large depression that is produced by conditioning of the intracellularly recorded IPSP (Figs. 52 and 53), and the lesser depression or even potentiation displayed by the extracellular positive field potentials under comparable conditions (Fig. 40). However, the large positive wave recorded just deeper to the parallel fibre volley would be very largely generated by the current flow from passive sources on the Purkinje dendrites to the more superficial sinks actively produced by the excitatory synapses (Fig. 42).

d) Comparison of inhibitory synaptic actions evoked by LOC. and J.F. stimulation.

In the Purkinje cell of Fig. 49 the IPSPs generated by J.F. stimulation were almost as large as the LOC. IPSPs and had a similar time course. In Fig. 51 this similarity of size and time course is exhibited by the inverted IPSPs from another Purkinje cell. These observations appear to be at variance with the regular finding that a fairly strong LOC. stimulation generated a high frequency repetitive

discharge of impulses from basket cells, whereas an equivalent strength of J.F. stimulation evoked the discharge of only one or two impulses (Table 1). In part this discrepancy can be accounted for if the repetitive basket cell discharge fails to evoke a commensurate increase in the IPSP because of transmitter depletion and/or receptor desensitization, factors which have been suggested above as explanations of the depression of IPSP production by a second LOC. stimulus. An additional and more attractive explanation is that the LOC. stimulation excites the basket cells occupying a narrow longitudinal band in the folium, whereas the J.F. stimulation would evoke the single or double discharge from much more widely distributed basket cells. Thus the IPSP produced in a Purkinje cell by J.F. stimulation would result from the summed effect of single or double impulses converging on it from many basket cell terminals, whereas with the LOC. stimulation there would be temporal summation of the synaptic action of many impulses in few basket cell terminals. The fact that a single action potential on a basket axon generates synaptic noise up to 5 mV would mean that spatial summation could indeed evoke a very large IPSP.

VII A) THE ANTIDROMIC INVASION OF PURKINJE CELLS

Although the dendritic tree of the Purkinje cells branches widely in the transverse plane of the cerebellar folia (Ramón y Cajal, 1911; Fox and Barnard, 1957); when a large population in a considerable area of a folium is simultaneously activated, the potential generated by the transverse components of the individual cells could be considered as cancelling, thus the resulting field potential would be equivalent to the arrangement in which each cell was merely a core conductor element perpendicular to the folial surface. This anatomical feature of the Purkinje cell population simplified the recorded potentials enough so that the extent and time course of antidromic propagation of impulses in the soma and dendrites of Purkinje cells can be revealed by a systematic study of the laminated field potentials produced by synchronous antidromic activation. Granit and Phillips (1956, 1957) studied the antidromic activation of Purkinje cells and focused their attention on the potentials produced by single cells, particularly on the "giant spikes" with their positive-negative configuration, and on the firing of impulses by Purkinje cells under diverse experimental conditions, and not on the details of the propagation of antidromic impulses and the field potentials produced thereby.

The antidromic propagation of impulses in Purkinje cells has been extensively investigated by intracellular and extracellular recording from

single cells and in particular, by a systematic study of the antidromic field potential profiles and of their modification by repetitive stimulation, and by the interaction with the inhibitory action on the Purkinje cells. This investigation has been performed on normal as well as on chronically deafferented cerebella, the latter in order to eliminate complications produced by stimulation of climbing and mossy fibres at the same time as the Purkinje cell axons. The chronic deafferented preparation also provides ideal conditions for studying the synaptic actions exerted by impulses in the axon collaterals of Purkinje cells (Ramón y Cajal, 1911; Jakob, 1928).

a) Antidromic responses of single Purkinje cells

The intracellular recording of antidromic spike potential in Purkinje cells resembles those of motoneurons and of most other neurons in amplitude, duration as well as in displaying an inflexion on the rising phase (arrows in Fig. 54A, B) which is now generally interpreted (Coombs, Curtis and Eccles, 1957; Terzuolo and Araki, 1961; Eccles, 1964, p.103-105) as being due to a delay in the antidromic invasion between the initial axonal segment (IS) and the somadendrite membrane (SD). However, C and D show that Purkinje cells differ from motoneurons in that even at the shortest response interval, there is seldom blockage of the antidromic IS-SD propagation and in general, only an increased IS-SD delay and a slight re-

duction in amplitude, which recovered with a small increase in the stimulus interval (E, F).

Since the antidromic responses of Fig. 54A-F were evoked by a stimulus of the cerebellar white matter in the juxta-fastigial region (J.F.), impulses in mossy and climbing fibres are likely to produce postsynaptic potentials with superimposed spikes after the initial antidromic invasion; hence these records cannot be used for the study of any potentials which may follow the initial spikes.

In the chronically deafferented cerebellum the potentials recorded extracellularly from single Purkinje cells following a J.F. stimulus are not complicated by the potential fields produced by climbing fibres and mossy fibres as in the normal cerebellum. In G-K the initial antidromic spike potential shows a slight inflexion in the falling phase which indicates a delay of the invasion, and which presumably corresponds to the potential recorded intracellularly in A and B. The extracellular responses to the second J.F. stimulation (H-K) also correspond to the intracellular potentials (D-F) in showing an increased delay at the presumed IS-SD inflexion (arrows), but at the shortest response interval there was no IS-SD blockage. It will be shown that the field potential evoked by an antidromic volley at very brief stimulus intervals gives evidence of impulse propagation in some component of Purkinje cells which has a specially rapid recovery.

b) Field potentials produced in the cerebellar cortex by juxta-fastigial stimulation.

The J.F. stimulation produces a complex potential field in the cerebellar cortex. For example in Fig. 55A,C at a depth of 330_u from the surface there is an initial diphasic spike (positive-negative) followed by a complex sequence of negative waves which begin at about 1.2 msec after the stimulus. The initial diphasic wave is so early that it must be produced by the propagation of impulses that are directly excited by the J.F. stimulus some 10 mm distant (Granit and Phillips, 1956; Matthews, Phillips and Rushworth, 1957). These fields could be produced in part by climbing and mossy fibres as well as by the antidromic impulses in the axons, the axon collaterals, the somata and the dendrites of Purkinje cells. On histological considerations it can be suspected that the antidromic invasion of the enormously extensive Purkinje cells would be dominant in generating the negative phase of the initial diphasic potential. The later potentials are presumably produced by the activation of the cerebellar afferents and their resulting postsynaptic effects. (See Page 56).

In B and D, a preceding parallel fibre stimulation caused almost complete suppression of the negative component of the initial diphasic potential, while the positive component was unchanged.

As reported above, LOC. stimulation evokes through basket and stellate cells a powerful postsynaptic inhibitory action on the somas

and dendrites of the Purkinje cells, hence the virtual suppression of the negative component can be attributed to the action of this inhibitory hyperpolarization in blocking the antidromic propagation into the Purkinje cell somata. There is no known way in which the conditioning stimulation of Fig. 55 B and D could depress propagation in mossy or climbing fibres. Evidently, therefore, the antidromic propagation of impulses in the Purkinje somata and dendrites generates almost all of the negative component of the initial diphasic potential in A and C.

The details of the antidromic spike potentials and of the inhibitory actions thereon are shown in the very fast records in E for depths from 80_{μ} to 630_{μ} . The diphasic potential continued to be large as superficially as 230_{μ} , but at more superficial levels (180 , 130 and 80_{μ}) there was a rapid and progressive decline in size, particularly of the negative component. It was surprising to find that from 630_{μ} to 230_{μ} the peak of the negative wave showed no detectable (less than 0.01 msec) trace of delay, such as would be expected for a propagating impulse. This fixed latency for the negative peak of the antidromic potential has been observed in all of the experiments recorded with a sufficiently fast sweep speed for significant measurement, as was the case in Figs. 55 and 56B. However, at more superficial levels the rapidly diminishing amplitude of the negative wave always exhibited a progressively delayed summit which in Fig. 55E

was 0.06 msec and 0.13 msec later at 180_u and 130_u respectively.

By superimposed plotting of the inhibited and uninhibited responses, the time courses and amplitudes of the inhibited components can be calculated by subtraction at each depth and are plotted in G. For levels from 630_u to 230_u the summit of the inhibited component occurred with the same latency, within 0.02 msec, there being only a small decline in size. However, there was a significant change in the onset at 230_u and superficially thereto, the inhibition caused some diminution of the initial positive wave. Also at 180 and 130_u the subtracted negative wave had a later summit, which corresponds to the later summit of the uninhibited response (E).

The potential waves in G may be recognized as being produced by antidromic invasion of the Purkinje somata and dendrites, for only this component of the initial spike response could be depressed by the inhibitory action evoked by a parallel fibre volley.

Similar investigations to those illustrated in Fig. 55 have been performed in several experiments and uniformly all have given similar results, though usually the degree of inhibition was less (cf. Figs. 58 and 59). At a depth of about 200_u the summit of the negative component of the diphasic wave has had the same latency as at 400 to 600_u , and its size has been reduced to 40 to 80% of that occurring at the soma level. There has been a rapid decline at the more superficial levels as well as a progressive increase in latency.

When the mossy and climbing fibres of the cerebellum were eliminated by the degeneration resulting from bilateral total pedunculotomy, J.F. stimulation always produced the largely simplified potentials illustrated in Fig. 56A. The triphasic spike potential at 800_u is attributable to the compound action current produced by the propagating impulses in the Purkinje cell axons. At depths from 400_u to 150_u there was an initial diphasic wave followed by a slow positivity having a total duration of about 15 msec. The initial diphasic (positive-negative) potential resembles that observed in the intact cerebellum except that the positive phase was relatively smaller. Experimental evidence has been searched to support the postulate that this diphasic wave is due to the antidromic propagation of impulses up the axons and into the somata and dendrites of Purkinje cells, and this identification has been corroborated by the demonstration of a similar diphasic potential in each of the ten successful experiments on the chronically deafferented cerebellum. In such cerebella only the axons of Purkinje cells would be available for conducting impulses from the J.F. region up to the cerebellar cortex. However, the small size of the initial positive component of the diphasic spike indicates that in the intact cerebellum much of this component is generated by impulses in mossy and climbing fibres. The manner of production of the slow positive wave of Fig. 56A will be considered in the discussion.

The very fast records from another deafferented cerebellum (B) allowed studying in detail the temporal relations of the extracellular spike potentials throughout the whole thickness of the cerebellar cortex. As shown in C (filled circles) the plotted measurements of the spike summits in B were synchronous within the limits of measurement at all depths from 600_{μ} , and synchronism was also observed for the antidromic spike potentials (crosses in C) set up by another J.F. stimulating electrode in this same experiment. However, this synchronism was not obtained for the rising phases of the spike potentials, which were progressively delayed along the antidromic pathway from 600_{μ} depth, as may be seen by the vertical line in B at 0.35 msec latency and by the plotted points (open circles) in C. Thus the early rising phase of the field potential exhibits a conduction velocity indicative of the progressive invasion of propagating impulses. An approximate conduction velocity of 5 to 10 m/sec is indicated. These findings of a progressively delayed rising phase and of a synchronized summit in the deafferented cerebellum exactly match those on the normal cerebellum (Fig. 55E). In Fig. 56 B and C, as seen in Fig. 55E, above 250_{μ} the antidromic spike potential declined rapidly in size and was progressively more delayed in its rising phase and summit.

In some experiments a complete reversal of potential at the most superficial levels was observed. For example, in Fig. 57A at 250 to 50_{μ} depth the antidromic field appeared as a small positive deflection.

There was also a gradual transformation of the slow positive wave so that the wave-form at 150 to 50_u was virtually a mirror image of that below 250_u. In A, the antidromic potential profile indicated that antidromic propagation ceased at an unusually deep level of the cortex - about 300_u; hence the prominence of the mirror reversal of the antidromic potential complex. Nevertheless, this reversal was also observed to occur to a smaller extent in several experiments where the antidromic propagation continued to the usual level.

c) Conditioning of the antidromic potential complex by a parallel fibre volley.

In Fig. 57B, parallel fibre stimulation produced in the chronically deafferented cerebellum a P-wave having a potential profile in depth resembling that of the normal cerebellum (Andersen, Eccles and Voorhoeve, 1964). For depths below 230_u the antidromic field potential in Fig. 57A had the same configuration as in Fig. 56A, a negativity followed by a slow positive wave. In Fig. 57B only the negative component was depressed by the preceding parallel fibre volley as in Fig. 55B, D, and the later slow positive wave suffered a large depression.

The sequence of potentials from 250_u up to 50_u in Fig. 57A displays the reversal of the antidromic potential complex (spike plus slow positive wave) that was referred to above. Fig. 57B shows

that the parallel fibre volley effected a re-reversal of this antidromic potential. A similar re-reversal was also observed in experiments on the normal cerebellum. An explanation of this effect will be given later.

Fig. 57C is a series resembling that of A, but recorded from another chronically deafferented cerebellum in which the antidromic complex became progressively smaller towards the surface and did not reverse. In D a parallel fibre volley had the usual inhibitory effect at depths from 200_u. By contrast, the much smaller antidromic spike complex from 100_u to the surface actually was facilitated by the parallel fibre volley. This facilitation at superficial levels was also observed in several experiments on the normal cerebellum. In both the normal and chronically deafferented cerebellum the facilitation was observed only when the recording was in proximity to the beam of excited parallel fibres.

Fig. 58C gives a typical depth profile of the action of a parallel fibre volley on the antidromic spike potential, specimen records being shown in A and B for the normal series and the series with the stronger inhibition (triangles in C). The antidromic field potentials for this normal cerebellum resembles in general those for the deafferented cerebella in Fig. 57, but of course the specimen records show the complex potentials which follow immediately the antidromic invasion (cf. Figs. 25, 55A-D). The degree of inhibition was much less than that of Fig. 55, which gives favorable conditions for comparing the in -

hibitory depressions of the antidromic spike potential at different levels of the cerebellar cortex. The depth profile (open circles) in C shows typically the absence of a large decrement in the uninhibited antidromic field until it was superficial to 200_{μ} . On the other hand, the inhibited field (filled circles and triangles) were quite small at 200_{μ} , there being a continuous severe decrement in the field amplitude from the deepest level. The depth profiles of the inhibitory diminution of the antidromic fields (D) show typically that, from a relatively small amount of inhibition at the deepest level, there was a progressive increase to a maximum at 250 to 200_{μ} depth.

For the most part the rapid decrement in the amount of inhibition superficially to 200_{μ} is attributable to the rapid decrease in size of the uninhibited response (C), but a contributory factor would also be the replacement of inhibition by facilitation at the most superficial levels, as illustrated in Fig. 57. The depth profiles of the uninhibited spikes for the experiment illustrated in Fig. 57 C, D are plotted in E for a testing interval of 25 msec, and in F there is the depth profile for the actual changes in the spike heights. In general this latter curve resembles those in D, but differs in that there is the clear facilitatory phase superficial to 150_{μ} .

The actions of a parallel fibre volley were tested in Figs. 57 and 58 at intervals of about 20 msec, which is approximately the time of maximum inhibition and facilitation. Fig. 59 shows a series of

specimen records of an antidromic field potential at a depth of 400_u in a normal cerebellar cortex (A) and at various intervals after a conditioning parallel fibre volley (B-F). At the two briefest testing intervals (B,C) there was no detectable inhibition, and in D to F the inhibition increased progressively with the increasing test interval. The full time course of the conditioning action of a parallel fibre volley is plotted in G from a comparable series of observations in another experiment. The inhibition was maximum at test intervals of 20 to 50 msec and full recovery did not occur until about 120 msec. Furthermore, G gives an indication of a slight and transient facilitation at about 7 msec test interval.

A similar brief and slight facilitation has been observed in several other experiments, and may be correlated with the intracellularly recorded potentials from Purkinje cells (Figs. 47, 48, 53), which sometimes showed an EPSP and even a spike potential just preceding the onset of the IPSP generated by a parallel fibre volley.

The prolonged time course of these IPSPs (Figs. 48, 49) matches the time course of the inhibition of the antidromic spike potentials in Fig. 59G. On the other hand the positive waves (P-waves) recorded extracellularly at the same time as the inhibitory curve (cf. Fig. 59C-F, and G, traced record) always had a very different time course. The P-wave onset was several milliseconds earlier, the maximum occurred early

on the incrementing phase of the inhibition and the P-wave actually terminated while the inhibition was still increasing. The complex problems involved in the attempt to relate the extracellularly recorded P-wave to inhibitory section on Purkinje cells will be treated later.

The curves in Fig. 59 for the time course of inhibition were obtained at a depth of 500_{μ} . In Fig. 60A the early parts of the inhibitory curves are plotted at depths of 300 and 500_{μ} in a chronically deafferented cerebellum. Just as in Fig. 59G, there was probably at 300_{μ} a small initial facilitation at 4 to 6 msec intervals, and there may even have been a trace in the 500_{μ} depth. However, at depths of 200 and 100_{μ} there was facilitation (B) resembling that illustrated in Figs. 57 B, D and 58E for testing intervals of about 20 msec. In Fig. 60B this facilitation had a duration similar to the inhibitory time courses at 500_{μ} depth in Fig. 59G. In Fig. 60B it can be seen that the inhibitory and facilitatory curves have an approximate mirror-image relationship. In the discussion, explanations will be developed for these mixed inhibitory and facilitatory actions of parallel fibre volleys.

d) Inhibitory action of antidromic impulses on the rhythmic responses of Purkinje cells.

Since Purkinje cells have been shown to have a direct inhibitory action on the cells of Deiters nucleus (Ito and Yoshida, 1964) and on

the intracerebellar nuclei (Ito, Yoshida and Obata, 1964), a similar synaptic inhibitory action could be expected for the axon collaterals of Purkinje cells. The chronically deafferented cerebellum gives opportunity for testing this inference. There is histological evidence that these collaterals contribute synapses to the large dendrites of Purkinje cells (Szentágothai, 1964a, 1964b, and personal communication; Fox, personal communication). However, testing by a second antidromic volley revealed no depression of Purkinje cells beyond the very brief depression which is explicable by refractoriness (Eccles, Llinás and Sasaki, 1965b). It could however be objected that the safety factor for antidromic invasion of Purkinje cells is such that weak synaptic inhibitions are ineffective as depressants. For this reason it is important to investigate the more sensitive index of inhibitory action that is provided by the spontaneous rhythmic discharges of Purkinje cells in a chronically deafferented cerebellum.

Fig. 61A gives a consecutive series of records in which single J.F. stimuli were applied during the rhythmic response of a Purkinje cell in a chronically deafferented cerebellum. In each of these five records the stimulus evoked an antidromic response marked by the arrows, and was followed by a silence of 52 to 146 msec, which is much longer than the longest cycle (35 msec) of the rhythm before the stimulation. However this silence was much briefer than the silence

produced by the inhibitory action of a single parallel fibre volley, the range being 325 to 380 msec for this rhythmic response.

In the deafferented cerebellum the mode of generation of the spontaneous rhythm is unknown; nevertheless the typical inhibitory action of the parallel fibre volley establishes that it is a sensitive indicator of postsynaptic inhibitory action on Purkinje cells. Hence it can be concluded that the antidromic volley in the Purkinje axons exerts a considerable inhibitory action on this Purkinje cell. The variability in the duration of the silence may be attributed in part to variations in the rhythmic mechanism itself, as disclosed by the irregularities in the rhythm before the silent periods, and in part to variations in a weak inhibitory mechanism (Fig. 61 B and C).

Other rhythmically discharging Purkinje cells in the deafferented cerebellum were tested for the inhibitory action of impulses in Purkinje cell axons, and inhibition has been demonstrated in 9, and it probably occurred in 4 other Purkinje cells, but their rhythmic discharges were too irregular to allow a convincing demonstration. It is important to recognize that this inhibitory action is very weak relative to the inhibition generated by parallel fibre volleys and mediated by **basket** and **stellate** cells.

The inhibitory action which may be postulated to be produced by impulses in the axon collaterals of Purkinje cells is so weak that it may have **but little** functional importance as a negative feed-back con-

of the Purkinje cells would be acting as sources in the control of Purkinje cell activity; nevertheless it is significant because it corroborates the general postulate that all the synapses made by the axonal branches of a neurone may produce the same synaptic transmitter and thus may have the same function: in this case there is inhibitory action by the synaptic terminals of the Purkinje cells in the intracellular nuclei (Ito, Yoshida and Obata, 1964) and in Deiters nucleus (Ito and Yoshida, 1964) as well as at the synapses of the Purkinje axon collaterals onto Purkinje cells. There is the further finding (Fox, 1962, personal communication; Szentágothai, personal communication) that Purkinje axon collaterals make many synapses on the somata and dendrites of basket and Golgi cells. Inhibitory action on these cells by such axon collaterals has also been demonstrated (Eccles, Llinás and Sasaki, 1965c). It is an example of a positive feed-back pathway by disinhibition (cf. Wilson and Burgess, 1962), the discharge of impulses by Purkinje cells inhibiting both the basket cells which inhibit Purkinje cells, and the Golgi cells which inhibit the excitatory granule cells.

B) DISCUSSION

- a) Interpretation of the field potentials produced by antidromic invasion of Purkinje cells.

Fig. 62A shows diagrammatically the flow of current at about 0.4 msec after the J.F. stimulation, when the somata and adjacent dendrites

of the Purkinje cells would be acting as sources in the external circuits for the sinks at the regions of the impulses. Since the circuit loops are so extensive vertically, relative to the transverse dimensions of the surface folium under investigation, the circuits correspond to the well-known features for parallel core conductors immersed in a conducting medium. At depths well below the superficial cortical layer (800_{μ} in Fig. 56A), the typical triphasic spike potential is observed as the volley of antidromic impulses passes by the tip of the recording electrode. This triphasic spike was rarely observed with a deeply placed microelectrode, presumably because the electrode was close to the cortical layers of deeper folia. At more superficial levels there is similarly an initial positive potential (Fig. 55), the time courses of these potentials being almost identical at depths of 500 to 200_{μ} , which would correspond to the somata and major dendrite. However, careful measurement of fast records discloses that the terminal phase of the positive wave becomes progressively later at more superficial levels, so that in Fig. 55 for example the crossing of the zero potential line occurs at 0.61 msec at 600_{μ} , and at 0.62, 0.635, 0.64, 0.665 msec at each successive 100_{μ} towards the surface. Hence the virtual absence of current flow over this range of levels during the first 0.4 msec is changed at 0.25 msec later to a considerable flow from sources in the more superficial dendrites to the sinks on the axons and somata of the Purkinje cells. There is similarly evidence for this current flow in Fig. 56B, C. This is,

of course, the current which flows as the Purkinje dendrites are being depolarized by electronic spread from the regions already invaded by the antidromic impulses.

It has been a remarkable finding that despite its later onset the summit of the extracellular antidromic spike potential is not appreciable (within 0.02 msec) later at the level of the Purkinje dendrites at 200_u than at the deeper levels of the somata and axons at 400 to 600_u (Figs. 55E, 56B,C). Furthermore, the negative antidromic field has been well maintained in size up to 200_u depth (Figs. 55E, 56A). More superficially the size rapidly declined and the summit was progressively more delayed, and close to the surface there was often a reversal of its polarity (Fig. 57A). The laminated potential field at the time of the antidromic spike potential suggests that the flow of current in the cerebellar cortex is as shown in Fig. 62B. The slight potential gradient at depths from 600 to 200_u indicates that there is little vertical current flow, but on the other hand from 200_u to the surface there is a large current flowing from sources near the surface to sinks as deep as 200_u, but not so much to deeper sinks, as is roughly indicated by the density of vertical current lines in B.

The sources at the superficial levels may actually be positive fields as in Fig. 57A, but more frequently are only relatively less

negative than at deeper levels as in Fig. 57C.

Despite the slightly later onset at more superficial levels, there was a virtually synchronous attainment of the peak negativity at depths of 600 to 200_u hence, it can be envisaged that the whole complex of soma plus large dendrites of a Purkinje cell tends to fire almost synchronously, presumably on account of so-called "trigger zones" which are located at strategic sites of the dendrites - perhaps at main branching sites - and which generate impulses at a lower threshold of depolarization, as has been postulated for example for hippocampal pyramidal cells (Spencer and Kandel, 1961b). These trigger zones may arise on account of background synaptic activation on the Purkinje cell dendrites. The distribution of sources and sinks in Fig. 62B shows that the most superficial levels of the Purkinje cell dendrites are heavily depolarized by electrotonic currents flowing into the larger dendrites. The small and later field potential at levels of 150 and 100_u could mean that there was at least some antidromic invasion after the instantaneous invasion up to about 200_u, but the rapidly diminishing spike indicates that for the most part impulse invasion would fail for dendrites at such levels, and there can be but little invasion superficial to the 100_u level. If the fine superficial dendrites are in this manner merely passively depolarized without spike initiation, it can be presumed that the small Purkinje dendrites that are

densely packed between the larger dendrites at deeper levels are not invaded antidromically either.

Over a wide range of levels (100_{μ} to 600_{μ}) in the chronically deafferented cerebellum the sharp negative potential signalling antidromic invasion of the Purkinje somata and dendrites is always followed by a slower positive wave (Figs. 56A, 57A,C) of 10 to 20 msec duration. At more superficial levels than 200_{μ} this positive wave declines parallel to the initial spike potential. There may even be reversal at the surface, which again parallels the reversal of the initial field (Fig. 57A). The laminar profile for this slow positive wave shows that the generating extracellular current corresponds to the diagrammatic illustration (Fig. 62C), which in fact is an exact reversal of that during the negative spike. It is remarkable that most experimental procedures link these two potentials together, so that a common mechanism of generation seems likely at least for part of the positive wave. In some cases, however, at depths of 500 to 300_{μ} , conditioning by a parallel fibre volley depressed the positive wave more than the initial negative field. If the positive waves were due to an active hyperpolarizing process, a differential depression of this kind would occur during the hyperpolarization of the IPSP produced by the parallel fibre volley. Thus the differential depression suggests the possibility that the positive wave might be in part due to inhibitory

synaptic currents, produced by the synapses which the axon collaterals of Purkinje cells give to the soma and to the main dendrites of the neighbouring Purkinje cells and possibly also to Golgi and basket cells.

By analogy with other nerve cells and axons (Hodgkin, 1964; Ito and Oshima, 1964) it can be assumed that in the membrane invaded by an impulse the initial phase of high sodium conductance leads on to a phase of high potassium conductance which rapidly restores the membrane potential; (this rapid restoration of membrane potential is illustrated by the intracellular spikes in Fig. 54A, B). In those regions of the dendrites not invaded by the antidromic impulse, such a rapid mechanism of restoration of the passively depolarized membranes is not present. The only intrinsic mechanism of recovery depends on the resting ionic permeabilities which will produce a recovery with a time course depending on the resting time constant of the membrane, which, similarly with other nerve cells (Coombs, Eccles and Fatt, 1955; Coombs, Curtis and Eccles, 1959; Spencer and Kandel, 1961a; Creutzfeldt, Lux and Nacimientos, 1964), may be assumed to be several milliseconds in duration. This recovery will therefore lag far behind the recovery of the activated regions of the membrane; and consequently there would be a reversal in the electronic current flow as the active regions recover to a higher level of polarization than the region invaded passively. This reverse current

could generate the slow positive wave.

An explanation can thus be given for the observed positive waves at all depths and for the correlation between the initial fast negative wave and the later slow positive wave. The latter part of this time course would give an appropriate measure (actually an underestimate) of the time constant of the membrane potential of the finest dendrites. This postulated diphasic current flow between the activated and passive components of a single core-conductor element has been demonstrated in the spinal motoneurone when the antidromic impulse in the initial segment failed to invade the soma-dendritic membrane (Coombs, Curtis and Eccles, 1957, Fig. 8).

It will be recognized that a great simplification has been introduced into this theoretical discussion by assuming that the small dendrites (the spiny branchlets) are concentrated in a layer superficial to the large invaded dendrites. Actually these small dendrites are also inextricably mixed up with the larger Purkinje dendrites at all levels of the molecular layer. However, the currents flowing in this mixed zone will be completely randomized, and so will not contribute to the general field potential. On the other hand, more superficially than 200_{μ} the large dendrites will be rapidly terminating by profuse branching; consequently there will be a progressive preponderance of small dendrites; hence there will be a laminated arrangement in the distribution of the currents generated by the individual elements, which gives justifi-

cation for the construction of the diagrams in Fig. 62A-C.

The potentials produced by antidromic propagation into the hypoglossal and oculomotor nuclei exhibit a configuration closely resembling those for Purkinje cells, there being an initial large negative field and a later slow positive wave. (Lorente de No', 1947, 1953). These potentials were interpreted on the basis of a closed neuronal field system which was developed by Lorente de No and which is based on an anatomical arrangement such that a concentration of neuron somatas in a locus is surrounded preponderantly by their dendrites, which is essentially the condition for the laminated arrangement of the Purkinje cells in which the dendrites are oriented to the free surface of the cortex. However, in part at least, Lorente de No's interpretation of the later slow positive wave differed radically from this one, it being postulated that it was due to continued invasion of the finer dendritic terminals which were concentrated in the peripheral boundary of the nuclei. In agreement with Lorente de No', during this positive wave the current would flow from the somata toward the dendritic terminals, however, this current would not be attributed to a delayed invasion of the dendrites, but rather to their delayed repolarization after their initial passive depolarization. It is not conceivable that the dendritic invasion by the antidromic impulses could continue for more than a fraction of a millisecond. For example,

when the dendritic invasion was facilitated by excitatory synaptic action on the Purkinje dendrites, the spike produced in that manner was not appreciably later than the somatic spike.

b) Inhibitory and excitatory synaptic action on antidromic impulse conduction.

When the inhibitory action evoked by a parallel fibre volley on the antidromic spike potential was at a moderate level, as in Figs. 56, 57, 58, and not the almost total inhibitory action as in Fig. 55, it provided information on the range of levels at which the inhibitory synapses were effectively acting on the Purkinje cells. It was an almost invariable finding of such experiments that the depth profile of the spike diminution reached a maximum at the 200 to 300_u level (Figs. 57, 58D,F). It was considerably less at 400_u and was progressively less at deeper levels. The time course of this inhibitory action (Fig. 59G) corresponds to the time course of the intracellular IPSP that is recorded in the Purkinje cell soma, and which was attributed by them to the inhibitory action of the basket cell synapses on the Purkinje cell somata. Such an IPSP would block the propagation of antidromic impulses into all those somata where the safety factor was low, and so would cause the observed depression of the antidromic spike potential.

If the blockage were due solely to an inhibitory action concen-

trated on the somata, it would be expected that there would be a rapid diminution of the number of antidromically propagated impulses at the soma and more superficial levels, i.e. at about 400_{μ} . If the impulses were able to propagate through such an inhibitory barrage at the soma level, it would be expected that they would continue to propagate in a normal manner up the dendritic trees. However, the depth profile of the antidromic spike potential indicates that the inhibition increased considerably in effectiveness during this phase of dendritic propagation, and in some cases there was even a sign of a second zone of effective inhibition at a depth of 250 to 200_{μ} .

In addition to this depressant action on the antidromic propagation of impulses, parallel fibre stimulation provided two demonstrations of facilitation of antidromic propagation at depths of about 300 to 400_{μ} . A small transient facilitation sometimes occurred at test intervals of about 6 msec (Fig. 59G), which was just before the onset of the inhibition; at superficial levels (200_{μ} to surface) there was often a facilitation or a re-reversal of the small antidromic spike (Fig. 57B,D). Both of these facilitation phenomena appear to be explicable by the direct excitatory action of parallel fibres on the Purkinje dendrites, and as would be expected, they were observed only when the recording electrode was close in to the beam of excited parallel fibres. So far as has been investigated (Fig. 60B), the time course of the antidromic spike

facilitation at superficial levels resembles that for inhibition at deeper levels. At these levels the slightly earlier onset of the monosynaptic excitatory action gives it a brief initial advantage over the disynaptic inhibition (Fig. 59B), and presumably it continues as a submerged background during the stronger inhibition phase.

Despite the inhibitory depression of the antidromic spike at deeper levels and its facilitation superficially (Fig. 60B, there continues to be a progressive decrement of the spike towards the surface, as is shown by the curve through the open circles in Fig. 58E. Inhibition accentuates the decrement at deeper levels (Fig. 58C, E), and the superficial excitatory action of the parallel fibres merely slows down the rate of the decrement at that level (Fig. 58E). These observations can be interpreted as due to the opposed actions of synaptic inhibition and excitation on antidromic propagation in the Purkinje dendrites: the net inhibition at deeper levels lowers the safety factor and causes blockage; at the more superficial levels the net excitation raises the safety factor above normal and so enhances above normal the dendritic propagation of the surviving impulses as in Fig. 57 B, D.

VII CONCLUDING REMARKS

The investigations described in this thesis have led to the development of a provisional hypothesis on the functional organization of the neurones of the cerebellar cortex. As shown diagrammatically in Fig. 63, the two inputs to the cerebellar cortex, the climbing and the mossy fibres, have an excitatory action onto different cortical neurones.

a) The climbing fibres are the way of termination of the olivocerebellar pathway (Szentágothai and Rajkovits, 1959) and establish several series of excitatory connections: with their own cells of origin in the inferior olive through axon collaterals (Ito, personal communications); with the cerebellar nuclei (Jansen and Brodal, 1954); and with the neurones of the cerebellar cortex. In the cerebellar cortex the most important input is onto the soma-dendritic region of single Purkinje cells (Ramón y Cajal, 1888) but in addition their axon collaterals make synaptic connections with the Golgi (Schiebel and Schiebel, 1954; Szentágothai, personal communications; Fox, personal communications) basket and stellate cells.

The collaterals in the inferior olive would excite other cells in the inferior olive and so would activate other climbing fibres by a positive feed-back action. At the same time the collaterals produce also activation of the cerebellar nuclei, and through the monosynaptic input to Purkinje cells, a disynaptic inhibition on these cerebellar nuclei (Ito, Yoshida and Obata, 1964). The activation of basket (Andersen, Eccles and Voorhoeve, 1964) and stellate (Eccles, Llinás and Sasaki, 1965b) cells

would produce an inhibitory action on the neighboring Purkinje cells. The excitatory action on Golgi cells would reduce the excitatory input through the M.G.R. (Eccles, Llinás and Sasaki 1965c)

The climbing fibre system can then be envisaged as having two possible functions: a) as a reading-out system to test the excitability of single Purkinje cells by introducing an all-or-nothing excitatory input to it (Eccles, Llinás and Sasaki, 1965a); or b) a short latency strong negative feedback to play an inhibitory action on the cerebellar nuclei which have been activated by the collaterals of the olivo-cerebellar pathway.

Since the climbing fibre system represents ontogenetically and possibly phylogenetically the first input to the Purkinje cells (Ramón y Cajal, 1911), it is possible that the second alternative might be more plausible. Some of the inputs to the inferior olive are illustrated in the diagram of Fig. 63; the activation of the inferior olive by inputs from the spinal cord can be seen in Fig. 12.

b) The mossy fibre input relays its activity to the granule cells (Ramón y Cajal, 1911) in the granular layer and to the dendrites of the Golgi cells (Ramón y Cajal, 1911). This input system is, in contrast with the climbing fibre input, very wide, each mossy fibre entering into contact with thousands of granule cells. The mossy fibre-granule cell relay (M.G.R.) is controlled by the inhibitory action of the Golgi cells, which is both a feedback inhibitory system by the parallel fibre-Golgi cell

synapse as well as a feed forward inhibitory system through the mossy-Golgi dendrites pathway (Szentágothai, 1965; Eccles, Llinás and Sasaki 1965a).

The axons of the granule cells establish an excitatory synaptic action on the dendrites of Purkinje cells (Andersen, Eccles and Voorhoeve, 1964) and an inhibitory action through their synapses on the basket and stellate interneuronal systems. The mossy fibre system is then an extensive and highly integrative system onto the dendrites of Purkinje cells and, on account of the large extent of the mossy fibres, it presumably plays the most important role on the mixing of the inputs from different sensory modalities on the cortex of the cerebellum. In the diagram of Fig. 63, an axon collateral is drawn (dashed line) from the mossy fibre input to the nuclei of the cerebellum; this pathway is the only part of the diagram having no experimental support as yet.

Finally, the activated Purkinje cells would have an inhibitory action onto Deiters nucleus (Ito and Yoshida, 1964) and onto the cerebellar nuclei (Ito, Yoshida and Obata, 1964) by means of their axon terminals. Their axon collaterals would have a direct inhibitory action onto other Purkinje cells (Eccles, Llinás and Sasaki, 1965b) and on the Golgi and basket cells (Eccles, Llinás and Sasaki, 1965c), and in this manner would act as a recurrent

facilitory system by disinhibition (Wilson and Burgess, 1962).

This hypothetical network can explain the inhibitory and facilitory actions which high and low frequency stimulation of the cerebellar cortex evoke on the decerebrate rigidity (Dow and Moruzzi, 1958). The low frequency stimulation would have mainly an inhibitory action on the Purkinje cells via the interneurons of the cerebellar cortex. High frequency stimulation would depress this inhibition and so produce direct activation of the Purkinje cells, and in this manner inhibition onto the regions generating the postural tonus.

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T A B L E 1

Properties of fourteen inhibitory interneurons that were accurately located in depth below the surface of the cerebellar cortex. The broken lines suggest their subdivision according to depths into superficial stellate, basket and Golgi cells

Depth (u)	Loc. Stim.		J. F. Stim.		Inferior olive stimulation		
	Range of latency (msec)	Maximal duration of dis- charges (msec)	Maximal frequency (impulse/ sec)	Latency (msec)	Spike number	Latency (msec)	Spike number
170	5.0-7.3	23	445	5.0	1	11.2	1
180	2.9-7.0	11	770	1.5	1	6.0	1
200	3.0-5.5	14	715	3.7	1	4.7	1

260	4.3-7.1	25	700	2.1	1	3.6	1
270	2.2-7.8	17	455	1.7	2	7.2	1
300	3.0-7.7	30	1,000	1.504.0	2-3	3.5	1
300	1.9-2.5	20	500	1.3	1	4.0	0-1
300	2.5-4.0	20	1,000	3.0	1	-	-
320	1.5-4.1	20	500	1.0-3.7	1-2	3.2-4.4	1
350	2.3-3.5	20	500	1.5-3.2	1	5.0-8.0	1
350	1.5-5.0	30	500	2.2	1	-	-

400	3.0-5.5.	27	400	2.1-2.7	1	8.0	0-1
500	2.7-9.0	35	1,000	1.2-3.0	2-3	9.0	1-2
500	3.0-4.0	30	1,000	2.0-3.0	4	4.0-7.0	0-1

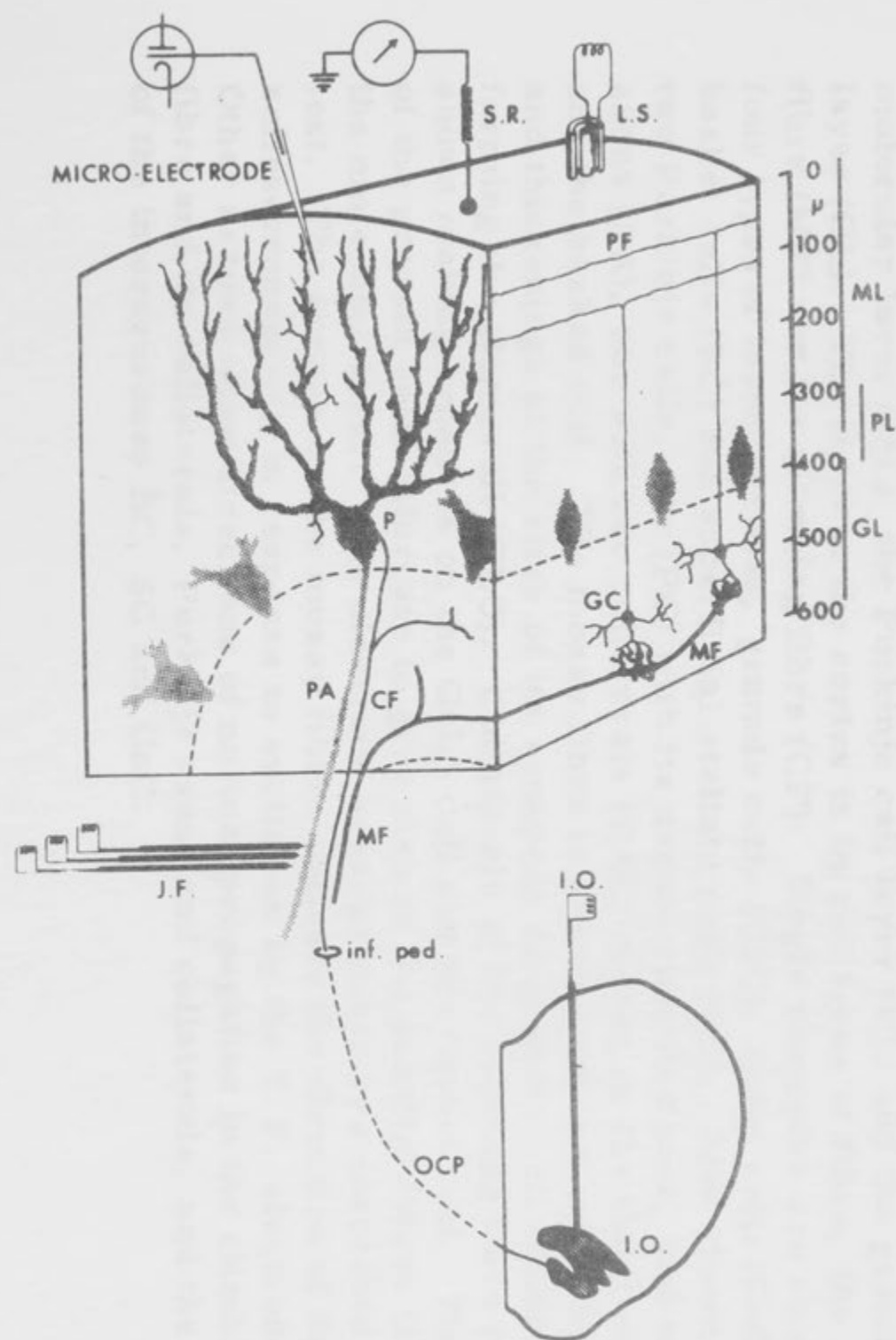


Fig. 1

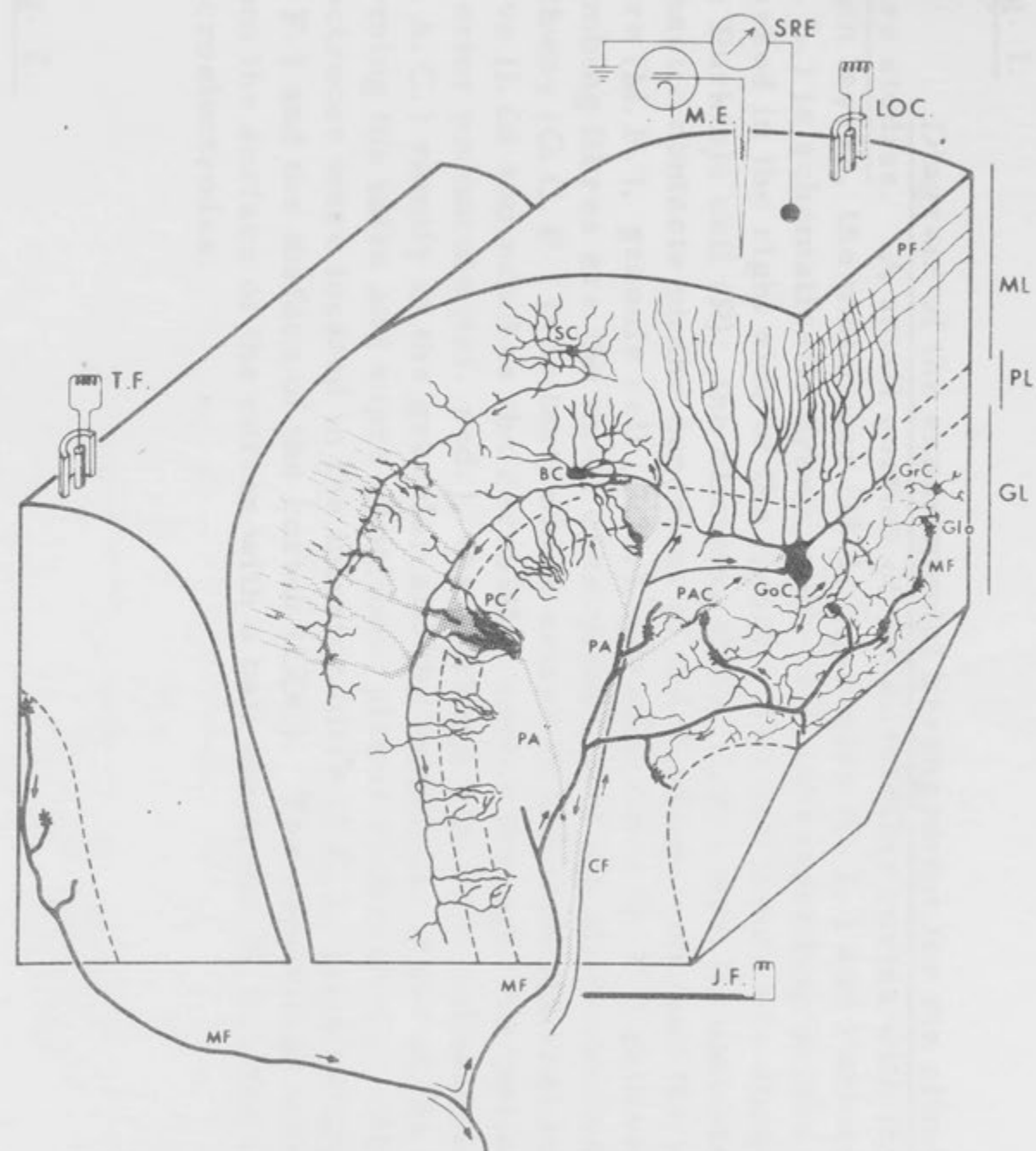


Fig. 2

Fig. 1.

Diagram of the experimental arrangement for the climbing fibre studies. A small portion of the cerebellar cortex with its three main layers, the molecular (M. L.) granular (G. L.) and Purkinje (P. L.) is schematically drawn, and their corresponding depths are marked in the right side of the figure. The two inputs are shown to the Purkinje cell (P): the climbing fibre (C. F.) with its extended synaptic contacts on the dendrites of the Purkinje cell; and the mossy fibre (M. F.), granule cell (G. C.), parallel fibre (P. F.) pathway. The climbing fibres are the mode of termination of the olivo-cerebellar pathway (O. C. P.) (see text) which arises in the contralateral inferior olive (I. O) and reaches the cerebellar cortex through the ipsilateral inferior peduncle (inf. ped.), the Purkinje cell axon collaterals (P. A. C.) ramify in the granular and the molecular layer of the cortex forming the infra and supra ganglionic plexus respectively. Stimulating electrodes were located in the inferior olive (I. O.), juxtafastigial region (J. F.) and the surface of the cortex (LOC). The recordings were done from the surface of the cortex with a ball electrode (S. R.) and in depth with microelectrodes.

Fig. 2.

Perspective drawing of a cerebellar folium to show the anatomical relationships of the different neurones and the experimental arrangements with respect to stimulating and recording electrodes. The cerebellar cortex is seen to be divided into three layers, the molecular layer (ML), the Purkinje cell layer (PL) and the granular layer (GL). The input to the cortex is by two types of fibre, the mossy fibre (MF) and the climbing fibre (CF). Single examples are shown of four types of interneurones, granule cells (GrC), Golgi cells (GoC), basket cells (BC) and superficial stellate cells (SC). Also shown are two Purkinje cells, one (PC) with its axonal ramifications, and both axons (PA), one with two collaterals (PAC) ending on the Golgi cell and the basket cell. The mossy fibre is shown with numerous branches and thickenings at the sites of its synapses on granule cell dendrites, so forming the glomeruli (GLO). Collaterals of the climbing fibre (CF) are shown making synapses on the Golgi cell and the basket cell. The axons of the granule cells bifurcate to give rise to the parallel fibres (PF) in the molecular layer. The electrode arrangements are described in the text. The arrows on the mossy fibres indicate the direction of the axon reflex propagation in response to excitation by the T. F. electrode. Other arrows show directions of normal propagation in the climbing fibre and its collaterals, Purkinje axons and collaterals, and the axons of the interneurones BC, SC and GoC.

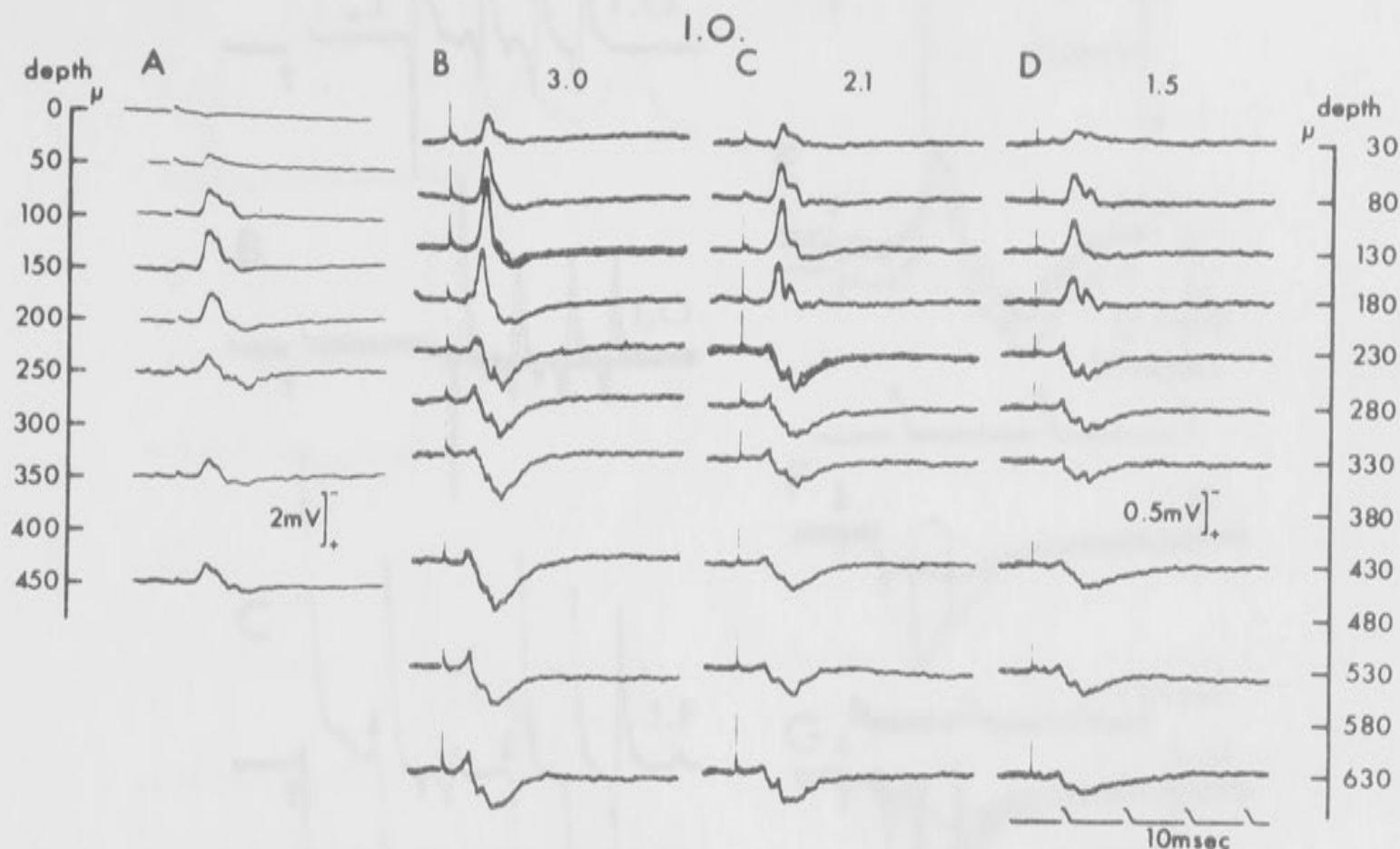


Fig. 3.

Laminar analysis of the field potential produced by I. O. stimulation in the contralateral cerebellar cortex. Two experiments are illustrated A (left depth scale) and B, C, D (depth scale to the right). Especially clear in record A is the difference in latency of the early negative component of the field at the different depths, which is interpreted to be the conduction time for the activity in the climbing fibres. Note the different voltage calibration for records A and B, C, D, but the time scale is the same.

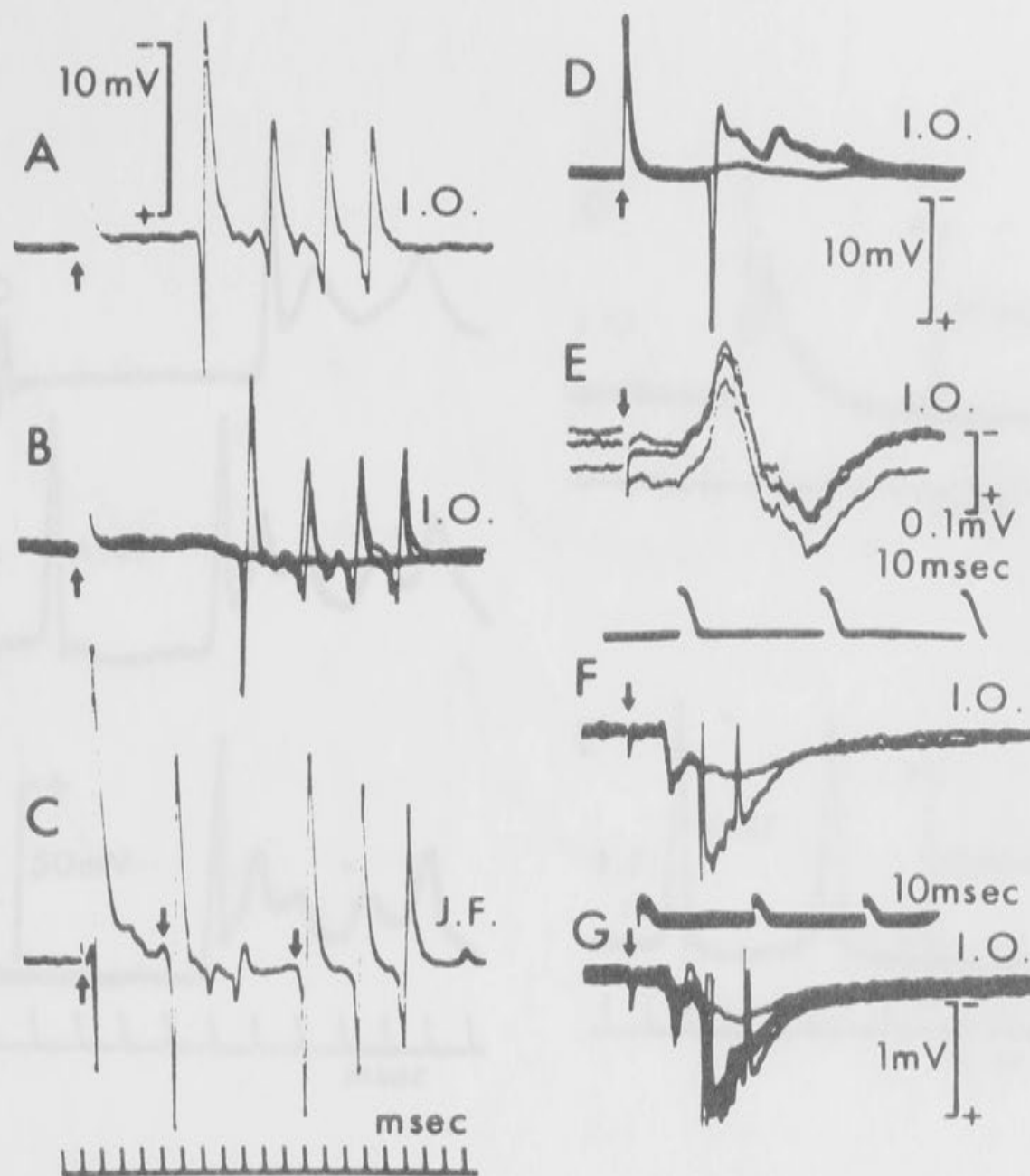


Fig. 4.

Inferior olive activation of Purkinje cells. An extracellular "giant" spike from a Purkinje cell is illustrated in A, B and C. In A and B the cell is fired by an I.O. stimulation. In B four superimposed sweeps show the all-or-nothing character of this response, two of the four stimuli being subthreshold. The spikes were so regular that they were superimposed. In B the latency was longer than in A because the stimulus was weaker. In C, following the antidromic invasion of the cell by J.F. stimulation, there are two sets of responses (down-going arrows); the first was produced by the direct stimulation of the incoming climbing fibre, the second to its reflex activation via the inferior olive. In D three superimposed sweeps show an all-or-nothing response of a Purkinje cell evoked by I.O. stimulation, and in E is a simultaneous recording from the surface of the cerebellar cortex (S.R. in Fig. 1). F and G are similar all-or-nothing responses of another Purkinje cell by I.O. stimulation, G illustrating the constancy of the response. For all records the first arrow signals stimulus artifacts.

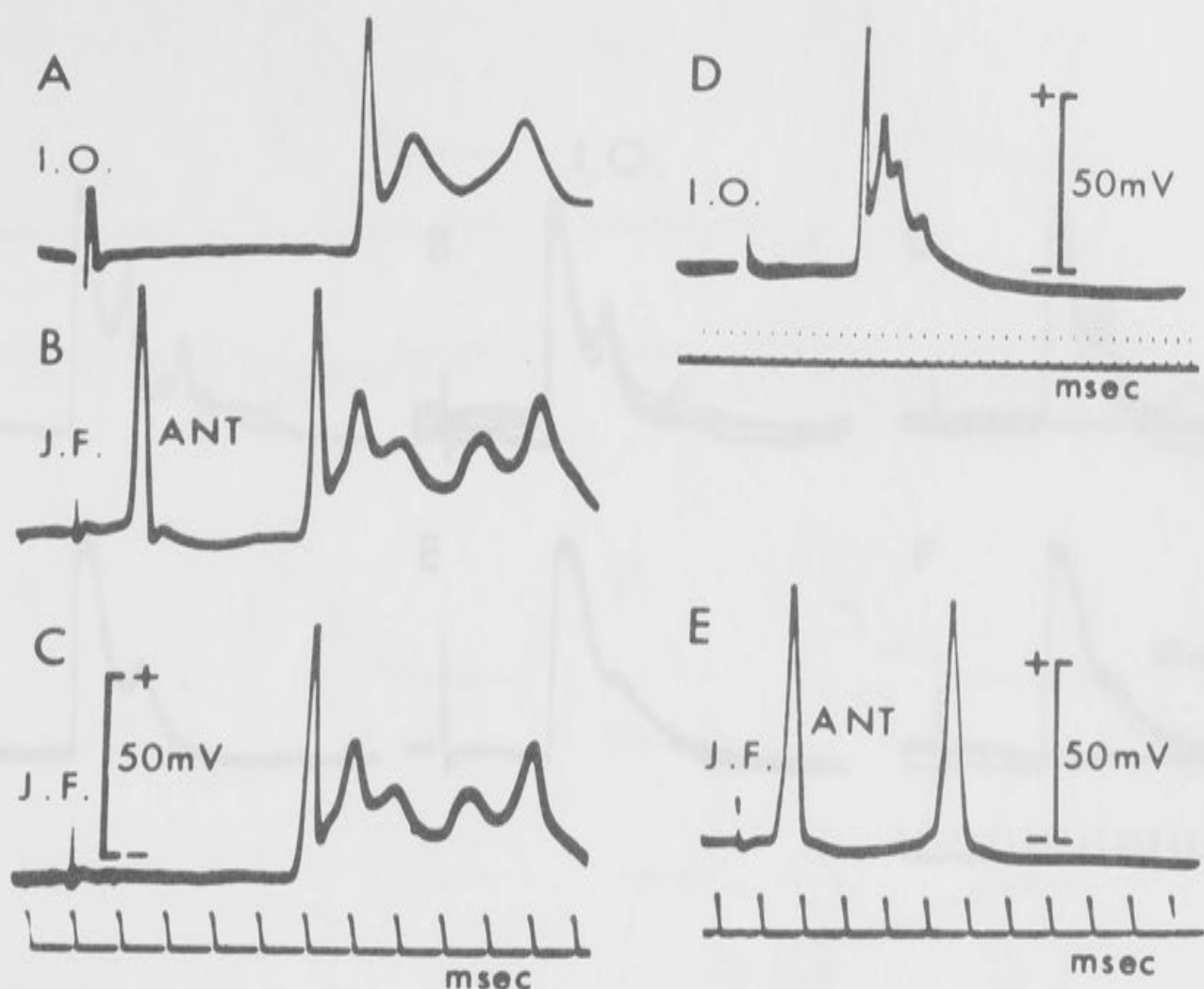


Fig. 5.

Intracellular records from Purkinje cells activated from the inferior olive and by juxta-fastigial stimulation. B and C responses were evoked by J.F. stimulation; in B the cell was antidromically activated, while in C the stimulus strength was subthreshold for antidromic invasion. In both there was a later spike followed by a prolonged complex depolarization that was virtually identical. I.O. stimulation in A evoked in the same cell a similar complex depolarization, the only difference being its longer latency. D and E are recording from another Purkinje cell, D being the intracellular response evoked by I.O. stimulation at a slow sweep speed and E shows the antidromic activation by J.F. stimulation. The second action potential in E is produced by mossy fibre activation through the granule cell - parallel fibre pathway.

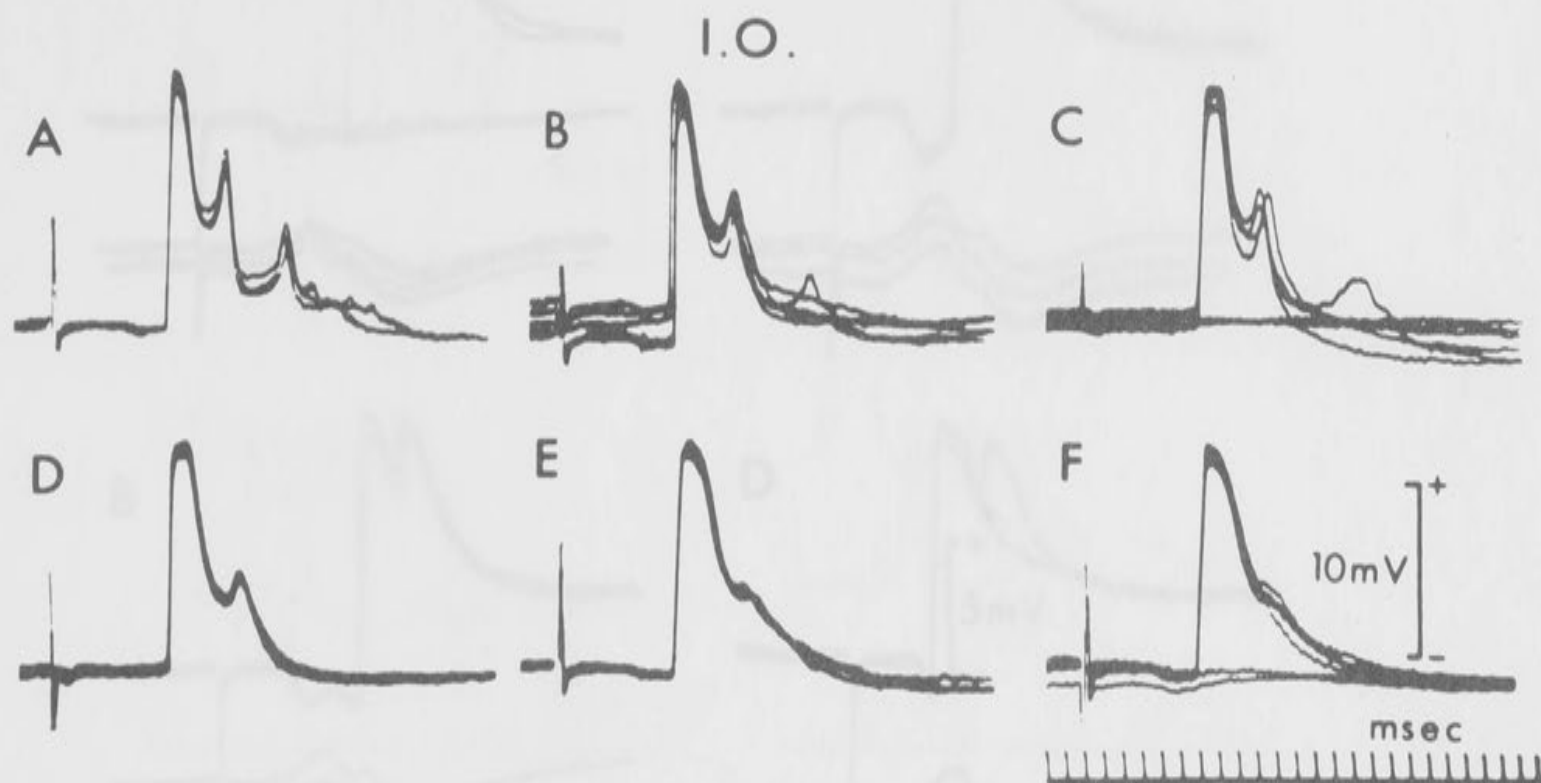


Fig. 6.

Intracellular records from a Purkinje cell evoked by I. O. stimulation. The sequence illustrates the different responses which a single climbing fibre produced in a slowly deteriorating cell. In A the synaptic activation produced a series of small responses on the declining phase of the large depolarization. These responses are well synchronized at A, and fade out as the cell deteriorates, so that at F there remains virtually only the early potential with a more or less smooth decay. Note the demonstration of the all-or-nothing nature of this response in C and F.

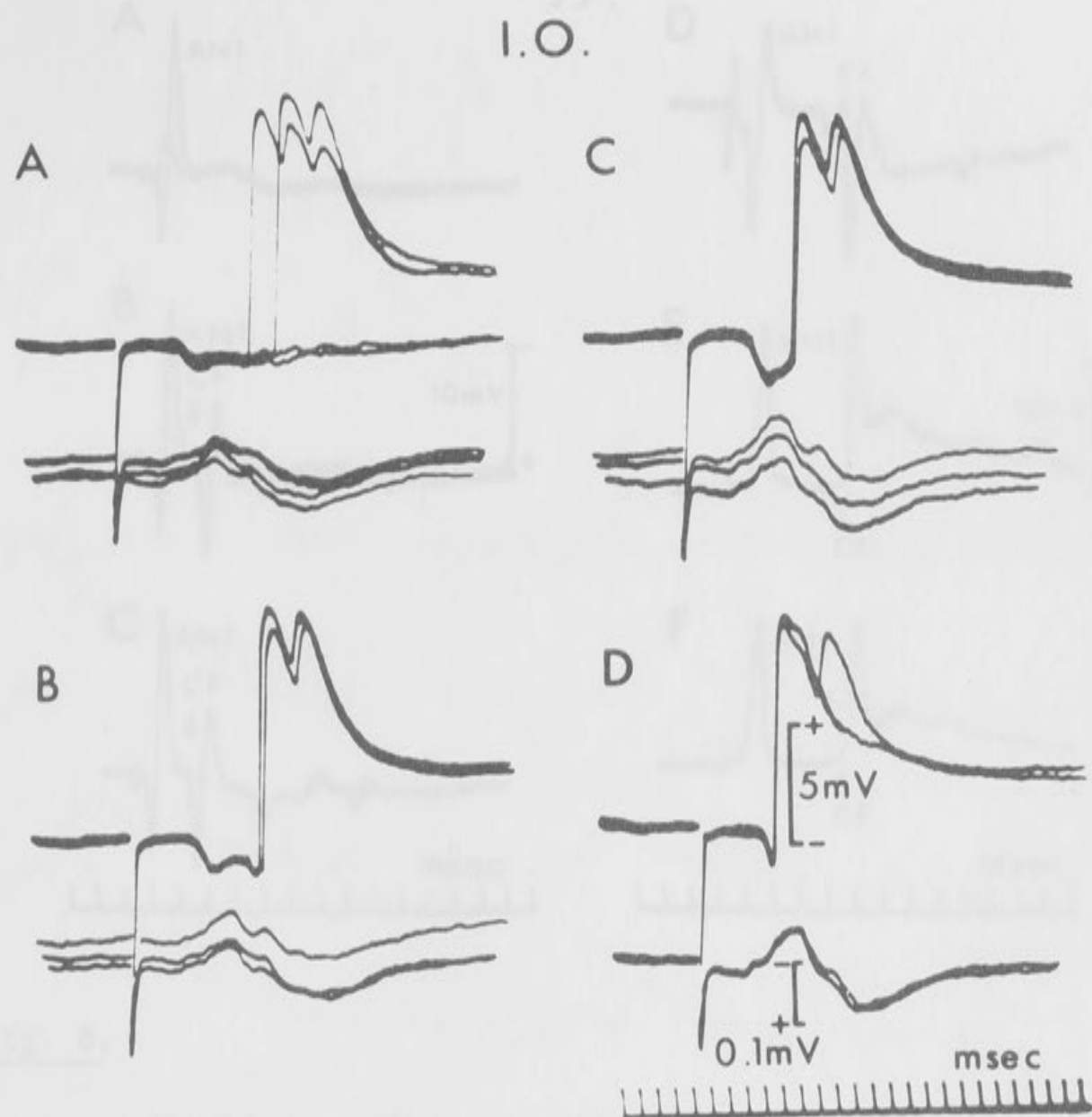


Fig. 7.

Intracellular record from a Purkinje cell showing different latencies for the synaptic potential evoked by I. O. stimulation. Lower records are the simultaneous potentials recorded at the surface of the cerebellar cortex with their own potential scale. The stimulus strength was increased from A to D. Note in A and D the all-or-nothing nature either of this synaptic potential or of its later component.

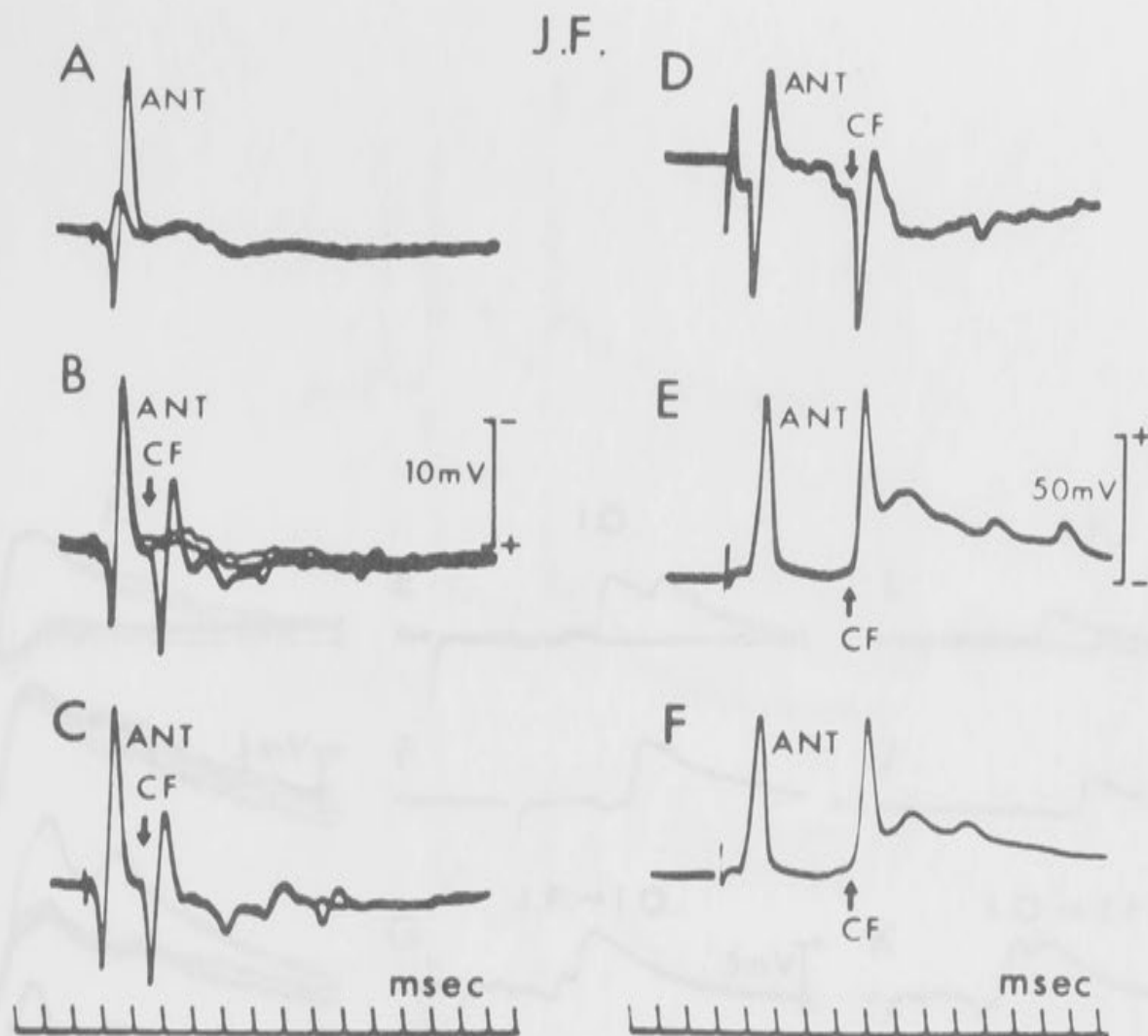


Fig. 8.

Purkinje cell response to J.F. stimulation. In A, B and C extracellular spikes were evoked by J.F. stimulation at different strengths. In A the stimulus strength was straddling threshold for the antidromic activation (ANT) of the cell. A small increase to the stimulus strength produced a second response (B) in an all-or-nothing manner (CF). In C a further increase in the stimulus strength evoked both responses on every occasion. In D are the extracellularly recorded responses from another Purkinje cell that also were evoked by J.F. stimulation and that closely resemble C. In E and F are intracellular records of the same cell showing, after the antidromic invasion, a second spike (arrow) followed by a long lasting depolarization (about 9 msec). The late potentials evoked by the J.F. stimulation in B to F are produced as indicated (CF) by the activation of a single climbing fibre. The potential scales of B and E are for the extracellular and intracellular records respectively.

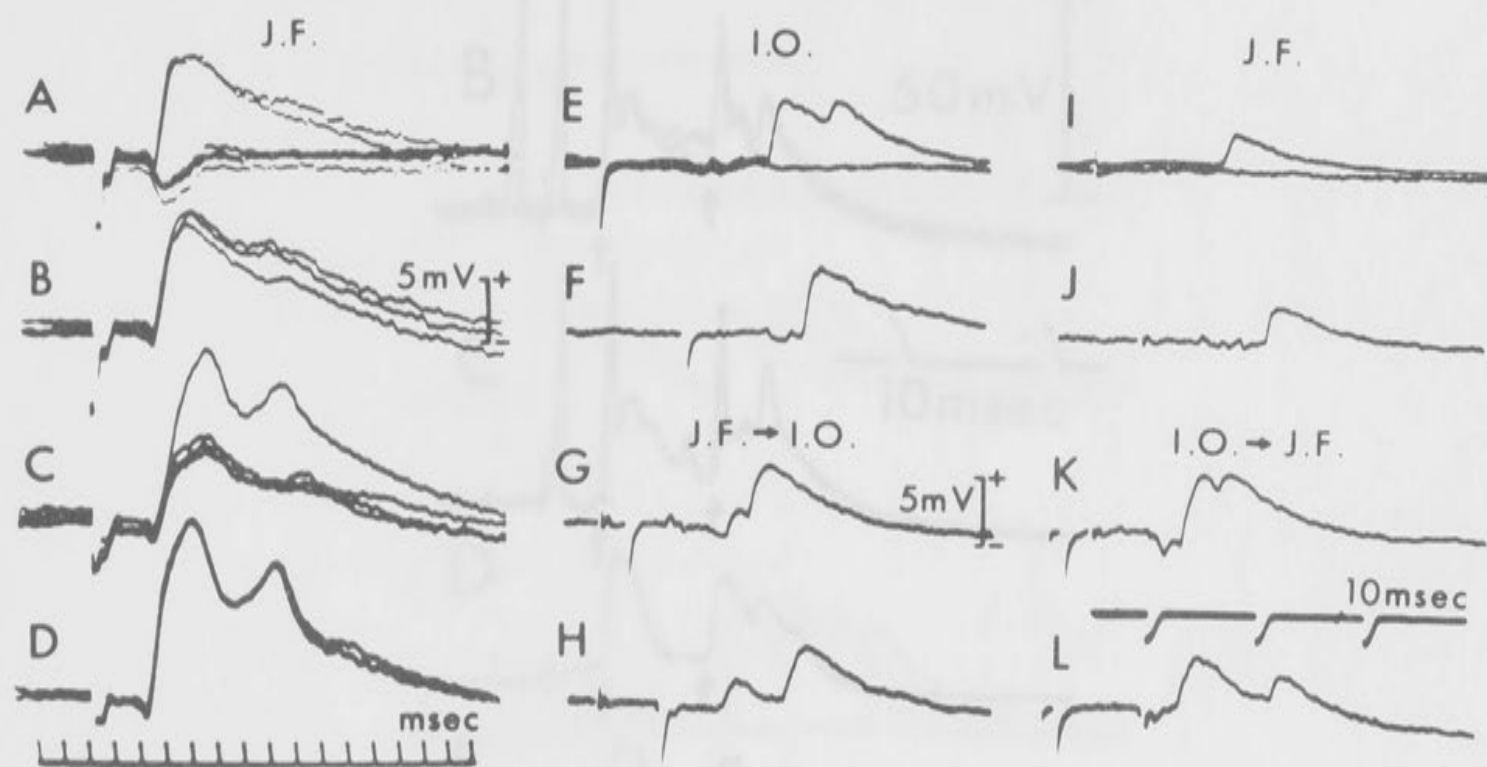


Fig. 9.

Intracellular Purkinje cell records showing double CF innervation.

A to D show four stimulation strengths for J. F. as described in the text. Records E-L illustrate responses of a Purkinje cell to two different climbing fibres, one being excited from the inferior olive (I. O. and E, F) and the other by juxtafastigial stimulation (J. F. in I and J) as described in the text.

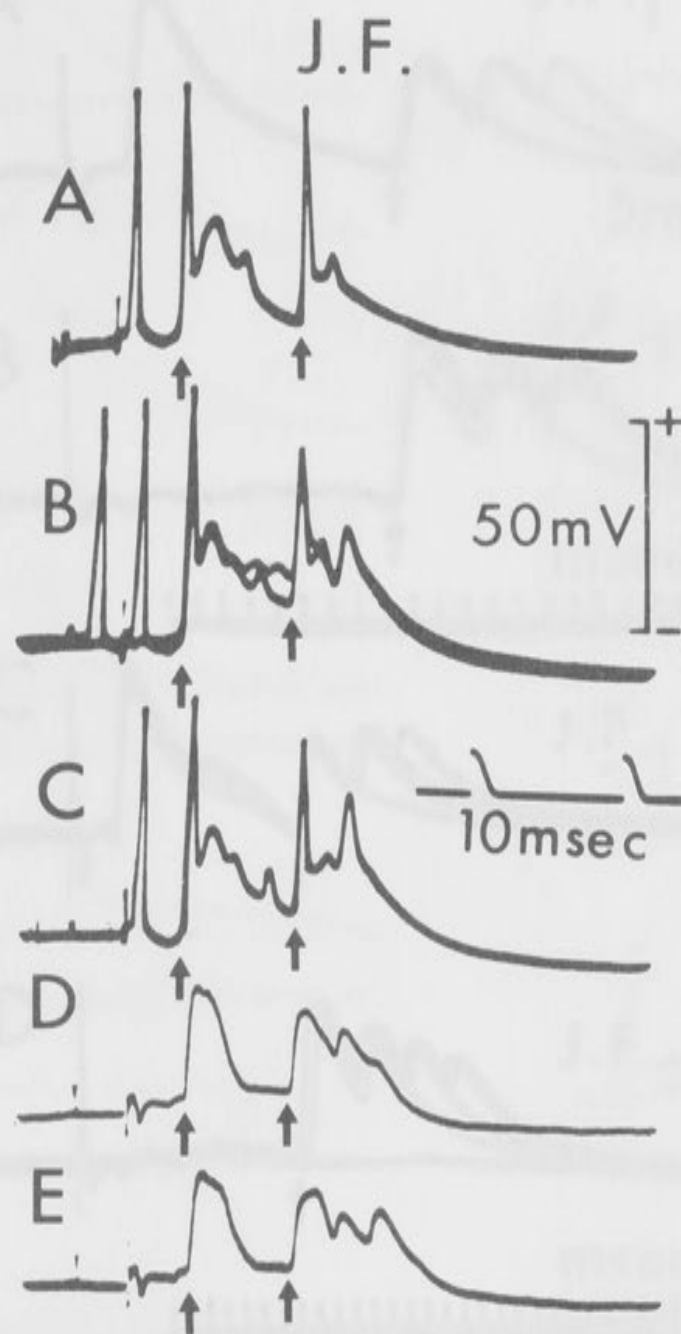


Fig. 10.

Intracellular Purkinje cell potentials evoked by reflex activation of a climbing fibre by J.F. stimulation. In A-C intracellular Purkinje spikes show an initial antidromic activation, then the direct CF responses at the first arrows, and the reflex CF response at the second arrow. In B a spontaneous spike prevented the antidromic invasion of the cell in one of the two superimposed sweeps. Records D and E were taken after the spike generation had deteriorated and show the direct and reflex CF responses at the same latencies as in A-C. Note potential and time scales.

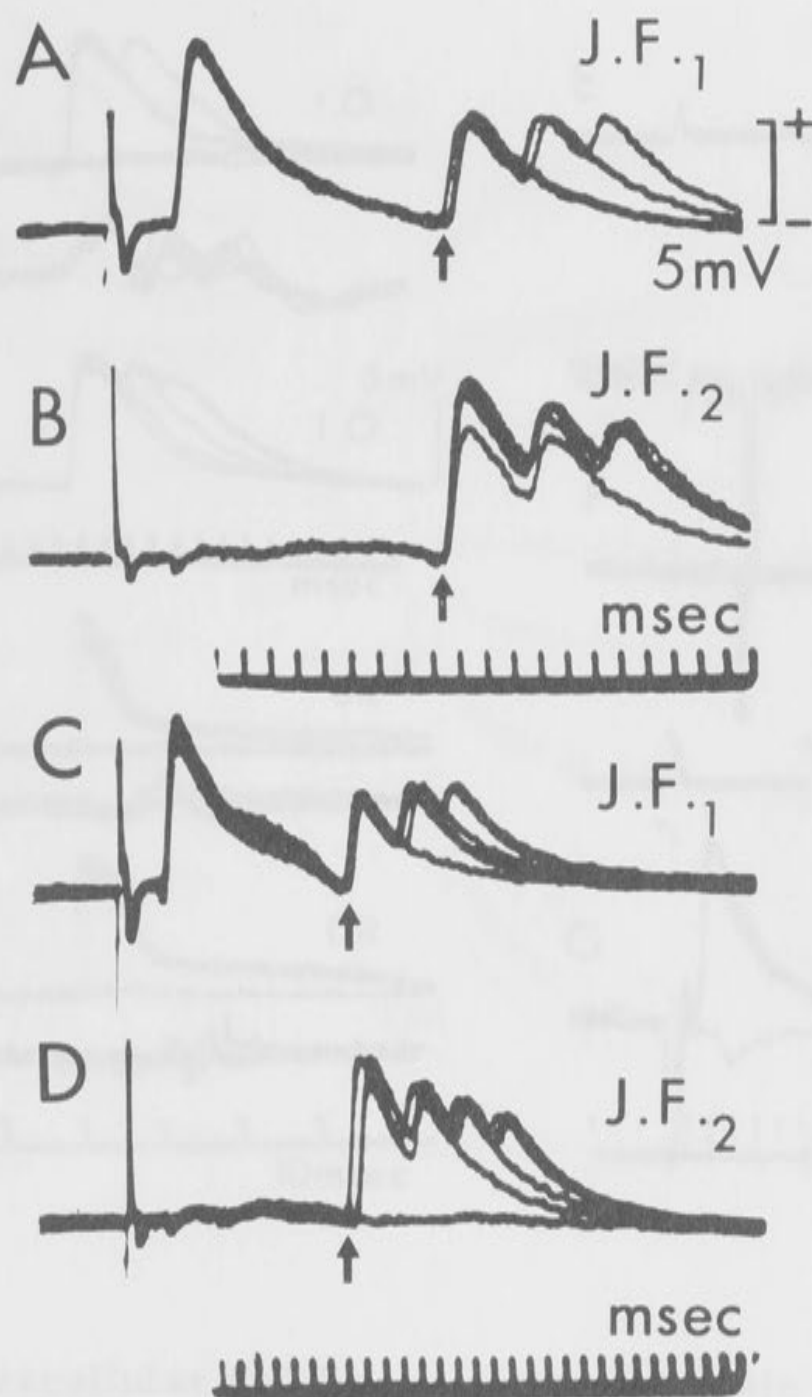


Fig. 11.

Intracellular Purkinje cell potentials produced by direct and reflex climbing fibre activation by J. F. stimulation. Record A shows a direct and reflex EPSP by the climbing fibre. In B stimulation by another J. F. electrode which did not stimulate this CF directly could nevertheless evoke the reflex activation of the CF at arrow. In C and D are similar potentials at slower speed.

The same amplification for A-D, but C, D at slower speed. Record C shows the typical intracellular climbing fibre spike together with the simultaneous record from the cerebellar cortex. In F the same CF spike complex was evoked in one hand by stimulation of the superficial cerebellar cortex (Fig. 10) in addition to evoking a single spike rising from the very same, at about 4.5 msec latency, which was evoked by the activation of the climbing fibre - granule cell - parallel fibre pathway. The latter slow and potential evoked by E and F. In D a CF all-or-nothing synaptic response was evoked in a Purkinje cell by local stimulation of the cerebellar cortex, the stimulating electrode (L.S.) in Fig. 1 being in close proximity to the Purkinje cell under study.

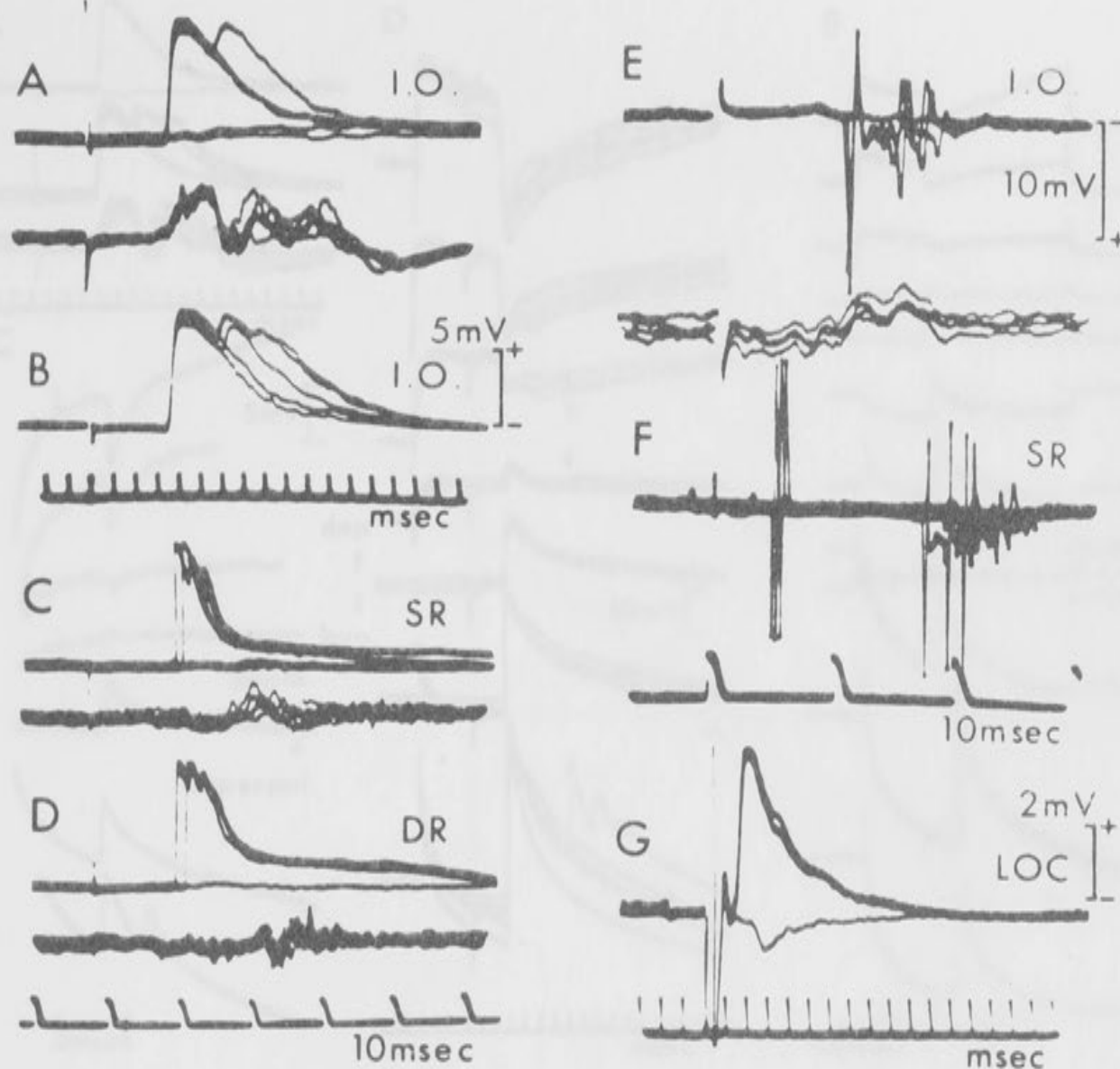


Fig. 12.

Extracellular and intracellular Purkinje cell responses evoked by climbing fibres activated in various ways.

A and B are intracellular potentials from a Purkinje cell showing the all-or-nothing CF synaptic potential evoked by I. O. stimulation, and the surface potential recorded simultaneously from the cerebellar cortex.

In C and D all-or-nothing CF synaptic potentials were evoked in the same cell by stimulation of the ipsilateral superficial (S. R.) and deep radial (D. R.) nerves respectively. Note the very prolonged synaptic depolarization indicating a prolonged transmitter action. The same amplification for A-D, but C, D at slower sweep.

Record E shows the typical extracellular all-or-nothing Purkinje spike complex evoked by CF activation by the I. O. stimulus, together with the simultaneous record from the cerebellar surface.

In F the same CF spike complex was evoked in the same cell by stimulation of the superficial radial nerve at 17 msec latency. In addition there was a single spike firing once in every sweep, at about 4.6 msec latency, which was evoked by the activation of the mossy fibre - granule cell - parallel fibre pathway. The same time and potential scales for E. and F.

In G a CF all-or-nothing synaptic response was evoked in a Purkinje cell by local stimulation of the cerebellar cortex, the stimulating electrode (L. S.) in Fig. 1 being in close proximity to the Purkinje cell under study.

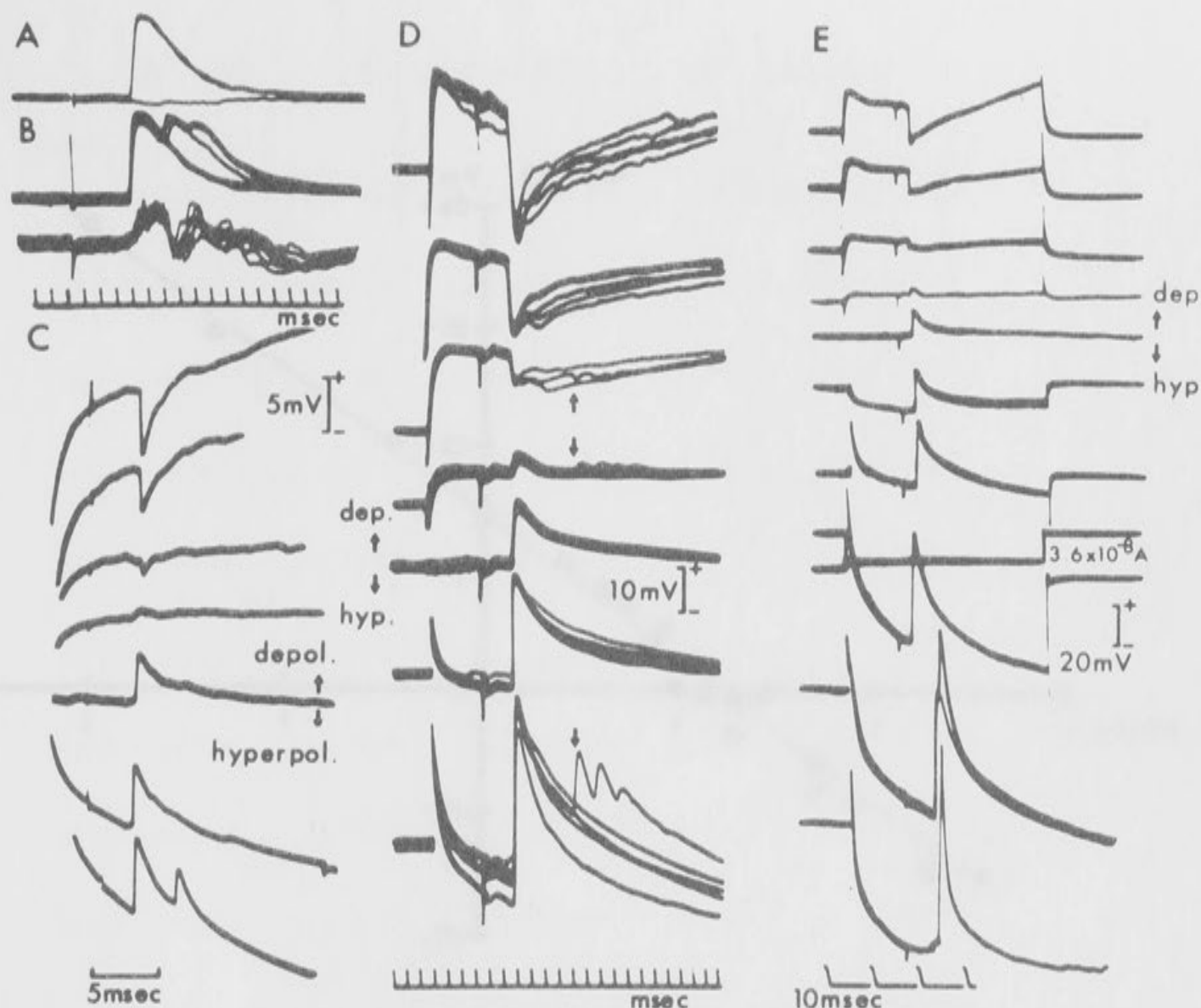


Fig. 13.

Intracellular Purkinje cell records of a CF synaptic potential modified by internally applied currents.

Records A and B show the CF-synaptic potential (EPSP) evoked by I. O. stimulation together with the surface recorded response in B.

In C an increase in the amplitude of the EPSP occurred when the hyperpolarizing currents steps were applied through the impaling microelectrode, while with depolarizing currents there was decreased and then reversal of the EPSP. The control synaptic potential is between the arrows. The same time and potential scales for A-C.

In D are superimposed records of the CF-EPSPs evoked in another Purkinje cell by J. F. stimulation. As in record C depolarizing and hyperpolarizing currents had the same effects on the CF-EPSP potential. Note that the reflexly activated repetitive CF response of this cell (marked by the three arrows) was altered by the applied current in the same way as the directly evoked EPSP.

Record E. shows a second series of current applications to the same cell as in D, and recorded at slower sweep speed and at lower gain. Reconstitution of the spike generating mechanism is observed when large hyperpolarizing currents were applied (lower two records). A current pulse $3.6 \times 10^{-8} \text{A}$ corresponds to the third potential record downwards from the control response, which as in C and D is between the dep. and hyp. arrows.

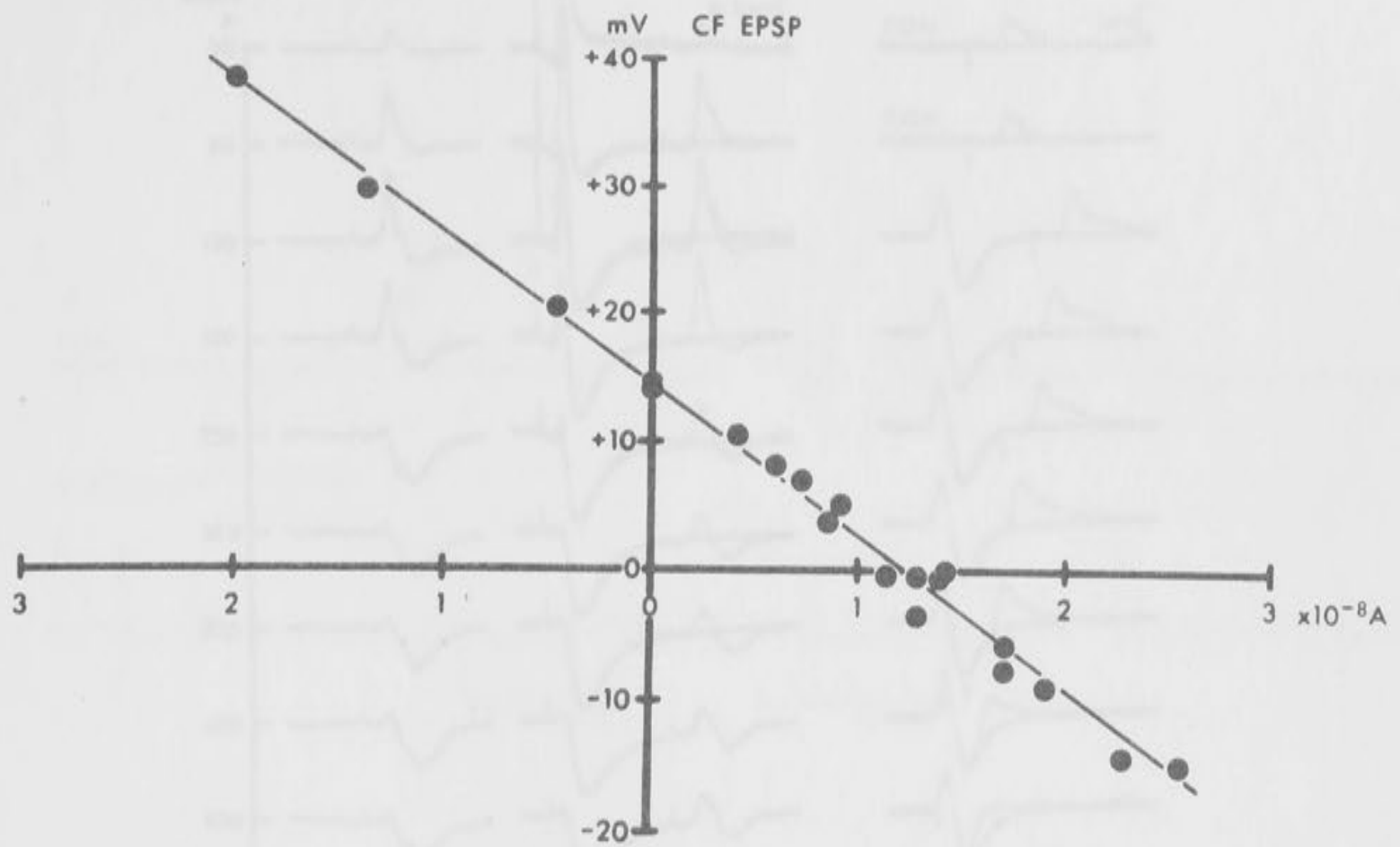


Fig. 14.

Plotting of the CF synaptic potential amplitudes (ordinates) against the intracellularly applied currents (abscissae). Note the straight line relation between the two.

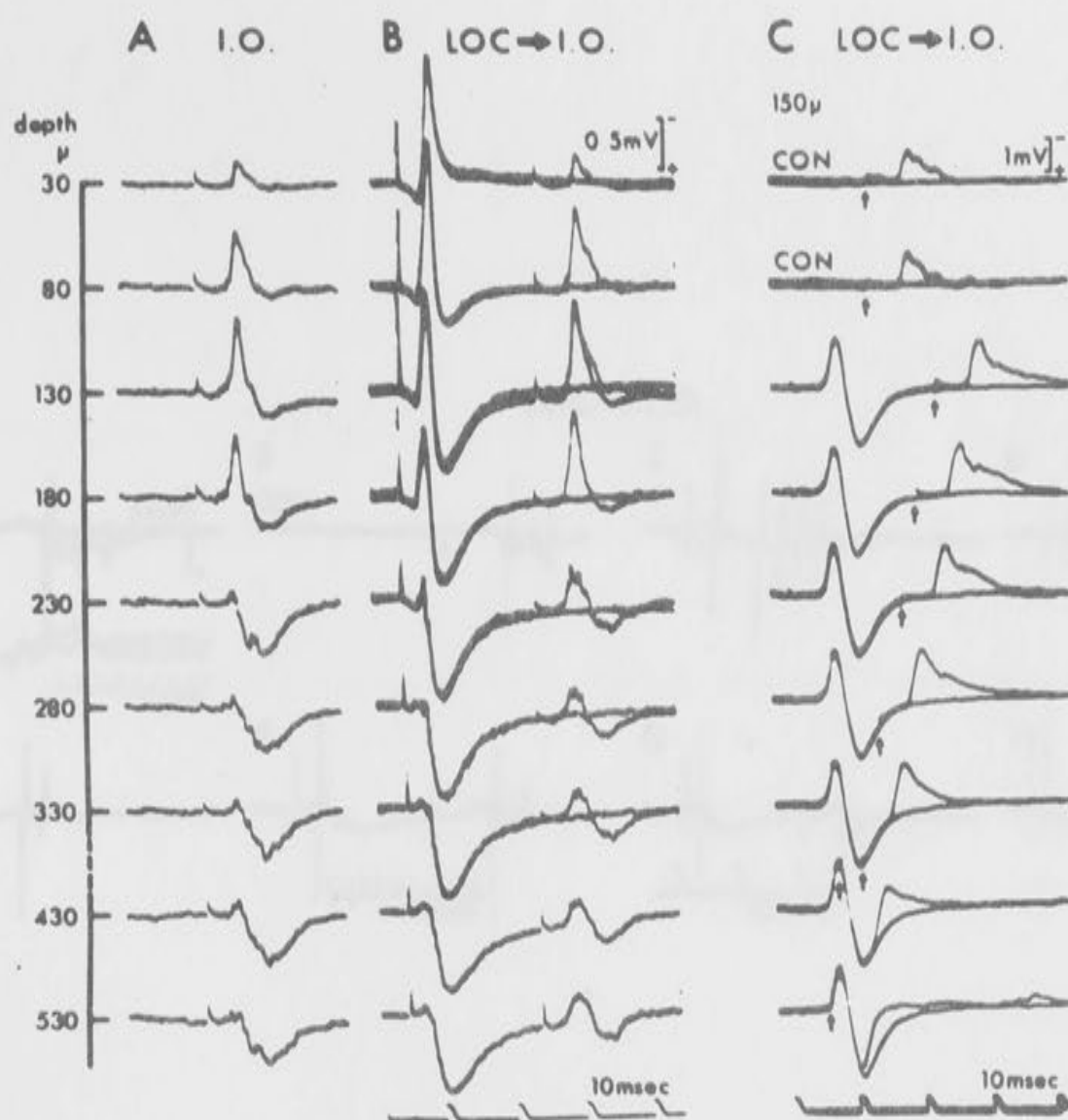


Fig. 15.

Field potentials produced in the cerebellar cortex by a single climbing fibre (CF) volley during the synaptic hyperpolarization of Purkinje cells.

In A, an inferior olive (I. O) stimulation evoked a typical potential field recorded at the different depths below the surface of the cerebellar cortex. When the I. O. stimulation was preceded by a conditioning local (LOC) stimulation in B, an increase of the negative component was observed. In C the CF potential field produced by an I. O. stimulation is shown at different intervals after a LOC stimulus and was recorded at 150 μ depth. The first two records give the control CF potential field (CON). The arrows mark the I. O. stimulus artifact. The voltage and time scales of B holds also for A.

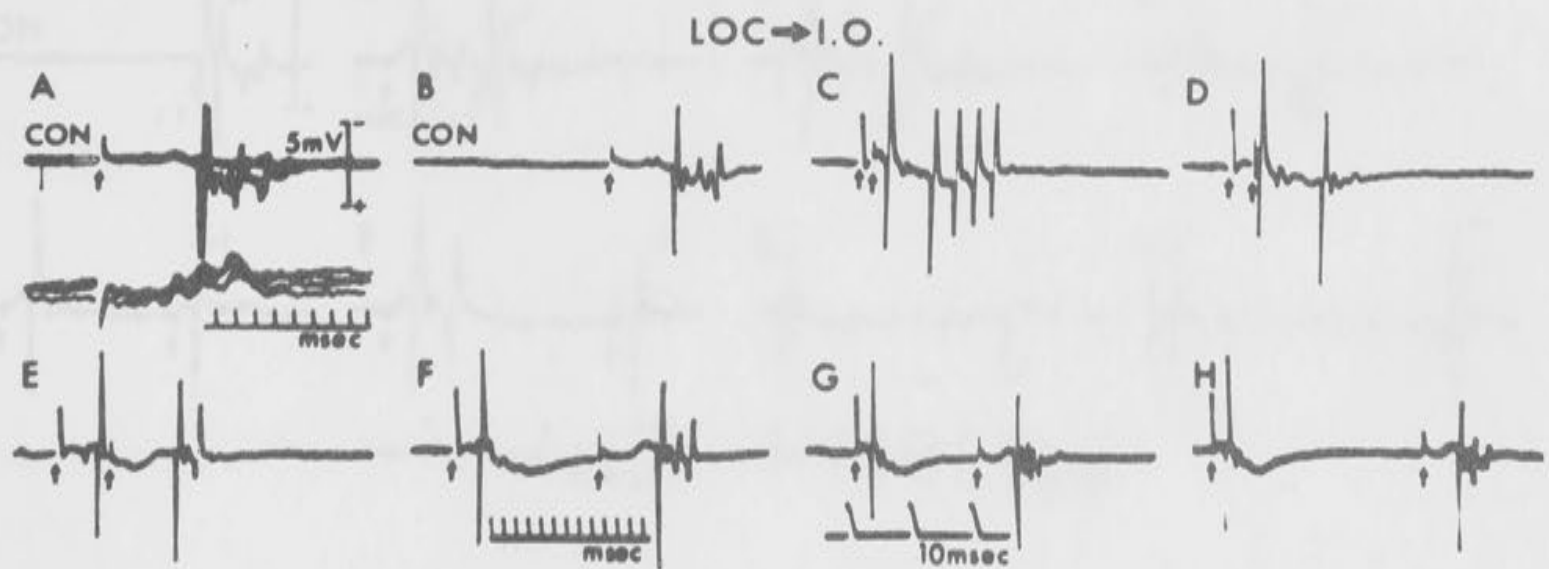


Fig. 16.

Extracellular records of a CF response from a single Purkinje cell evoked after a conditioning LOC. stimulation.

The first two records, A and B, are the control (CON) extracellular spikes of a single Purkinje cell produced by synaptic excitation by a single CF impulse. Record A shows the all-or-nothing character of this response, and the surface potential recorded simultaneously by the L. R. electrode (see Fig. 1) In C-H there was partial inhibition of the CF response of the Purkinje cell by a conditioning LOC stimulation. Records A, B-F and G-H were taken at different sweep speeds, but the voltage calibration was the same for all. The arrows mark the stimulus artifacts.

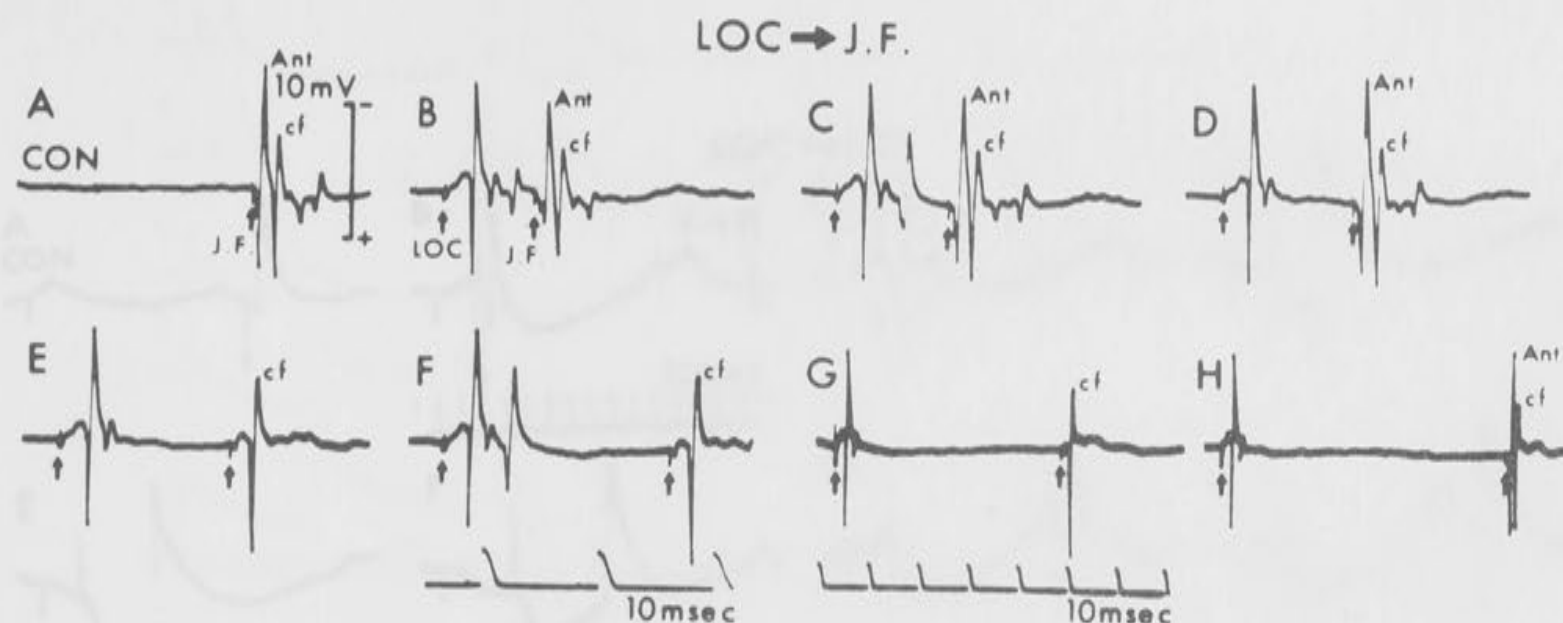


Fig. 17.

Extracellular records of the CF responses of a single Purkinje cell evoked by J.F. stimulation, and conditioned by a LOC stimulation.

A gives the control (CON) extracellular responses evoked in the Purkinje cell by a J.F. stimulation, showing the initial antidromic spike (Ant) and the later CF response (cf). In records B-H the J.F. response was conditioned by a preceding LOC stimulation which itself produced extracellular spikes due to the excitatory activity of the parallel fibres on to the Purkinje cell. The antidromic invasion and the late small spikes of the response were blocked from E to G. At H the antidromic invasion reappeared. Records G-H were taken at slower sweep speed. Arrow indicate stimulation artifacts.

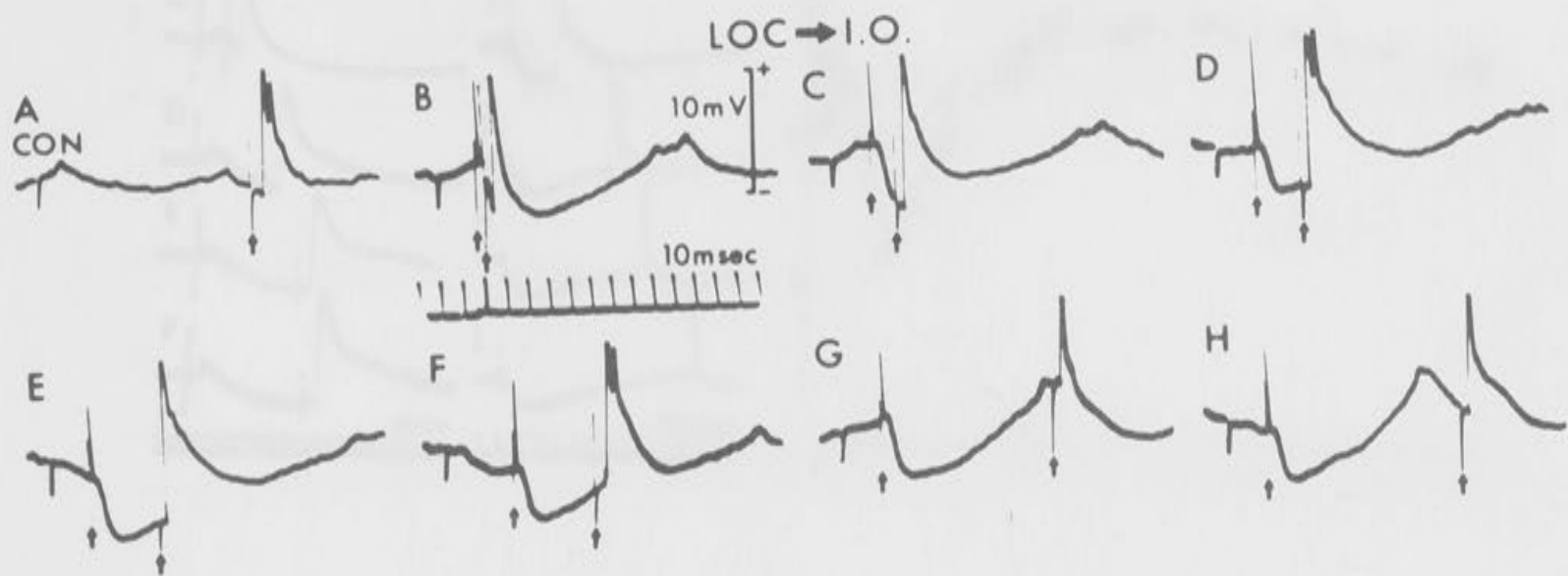


Fig. 18.

Intracellular records from a Purkinje cell showing the interaction between a CF EPSP and the IPSP evoked by parallel fibre stimulation.

A shows control EPSP evoked by I. O. stimulation at the arrow. Records B-H give changes in amplitude and time course of the I. O. evoked EPSP at different intervals after the initiation of the IPSP. All records are at the same time and voltage scale. Arrows indicate stimulation artifacts.

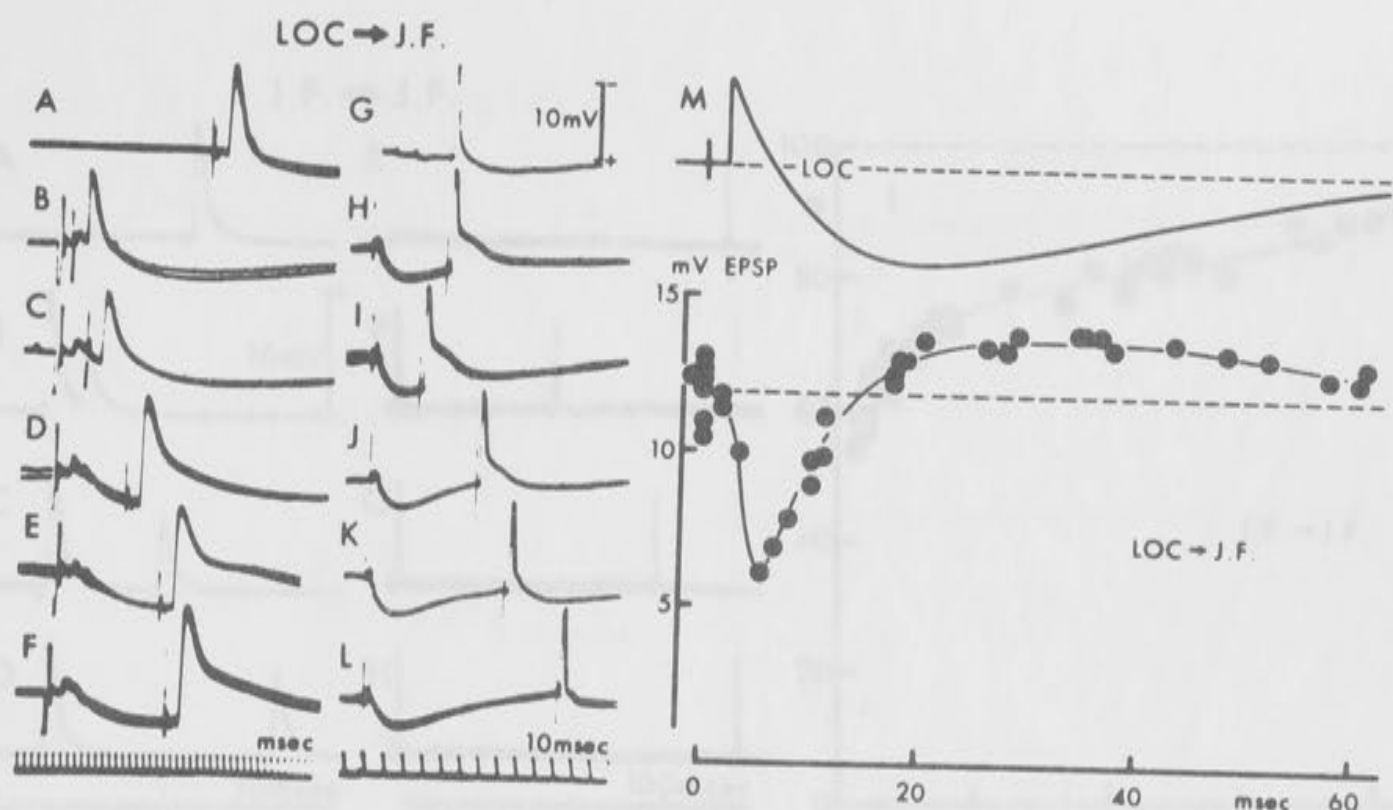


Fig. 19.

Intracellular records of a Purkinje cell showing the time course of the interaction between the IPSP evoked by parallel fibre stimulation (LOC) and the CF EPSP evoked by J. F. stimulation.

Record A shows the control EPSP evoked by J. F. stimulation.

In B-F, the EPSP was superimposed at different intervals after the initiation of the IPSP.

H-L give another series from the same cell taken at slower sweep speed, G being the control EPSP.

M is a plot of the time course of the interaction between the CF EPSP and the LOC IPSP. The records were taken from another series from the same Purkinje cell as that illustrated in A-L. The amplitude of the EPSP in millivolts (ordinate) is plotted against the interval in milliseconds between the LOC stimulation and the J. F. stimulation. The drawing in the upper part of the figure shows the time course and amplitude of the synaptic potentials evoked in that Purkinje cell by the LOC stimulation, with the same time and voltage scale as for the plotting underneath. The initial parallel fibre EPSP is followed by the prolonged IPSP produced by the inhibitory interneurons.

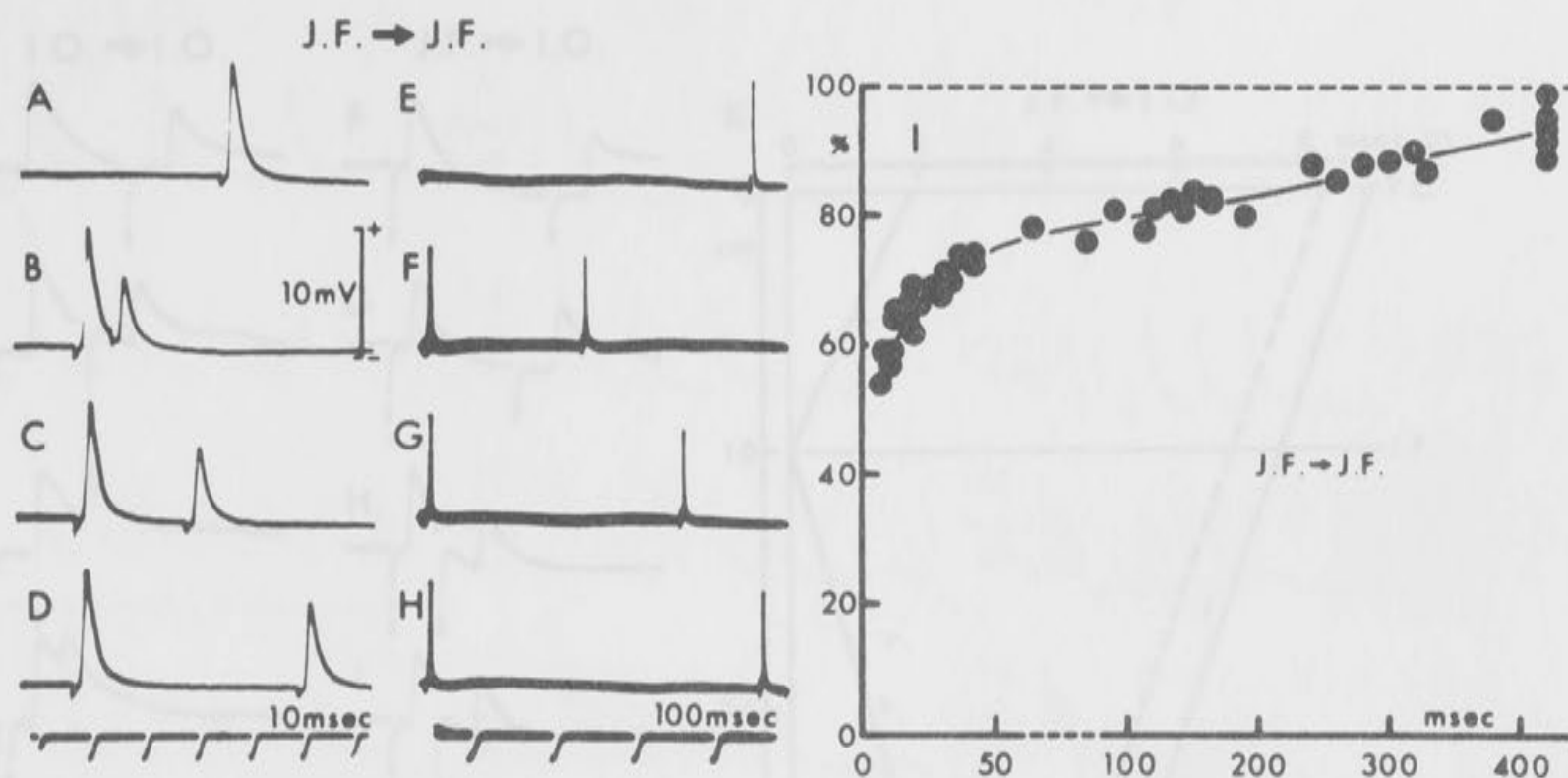


Fig. 20.

Intracellular records from a Purkinje cell showing interaction of two CF EPSPs evoked by J. F. stimulation.

A and E controls. B-D and F-H show interactions at successively increasing intervals between the two J. F. stimulations. The records were taken at two different sweep speeds. In I the amplitudes of the second EPSPs in percentages of the control record (ordinate) were plotted against the intervals in milliseconds (abscissa). Note the time scale was changed for the abscissa at 70 msec.

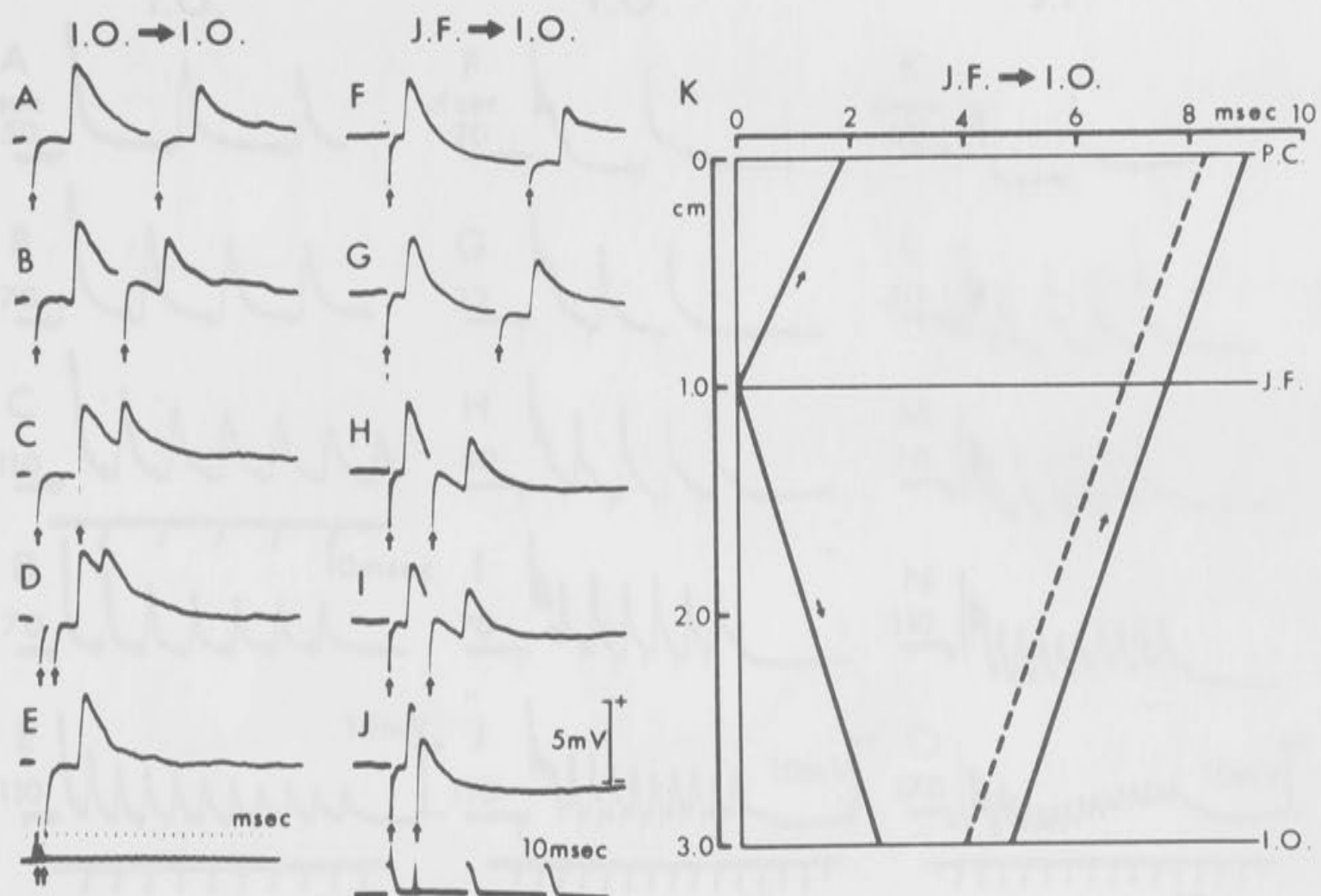


Fig. 21.

Intracellular records from a Purkinje cell showing EPSP produced by two CF impulses evoked by I. O. and J. F. stimulation. In A-E both CF impulses were produced by I. O. stimulation, refractoriness preventing the generation of the second CF impulse in E. In F-J impulses in the same climbing fibre were produced, firstly by J. F. then by I. O. stimulation. It failed in J. Arrows indicate stimulus artifacts. Diagram in K is the plotting of a collision test between the J. F. and I. O. stimulation, the time in msec (abscissa) is plotted against distance between electrodes in cms (ordinate). See text.

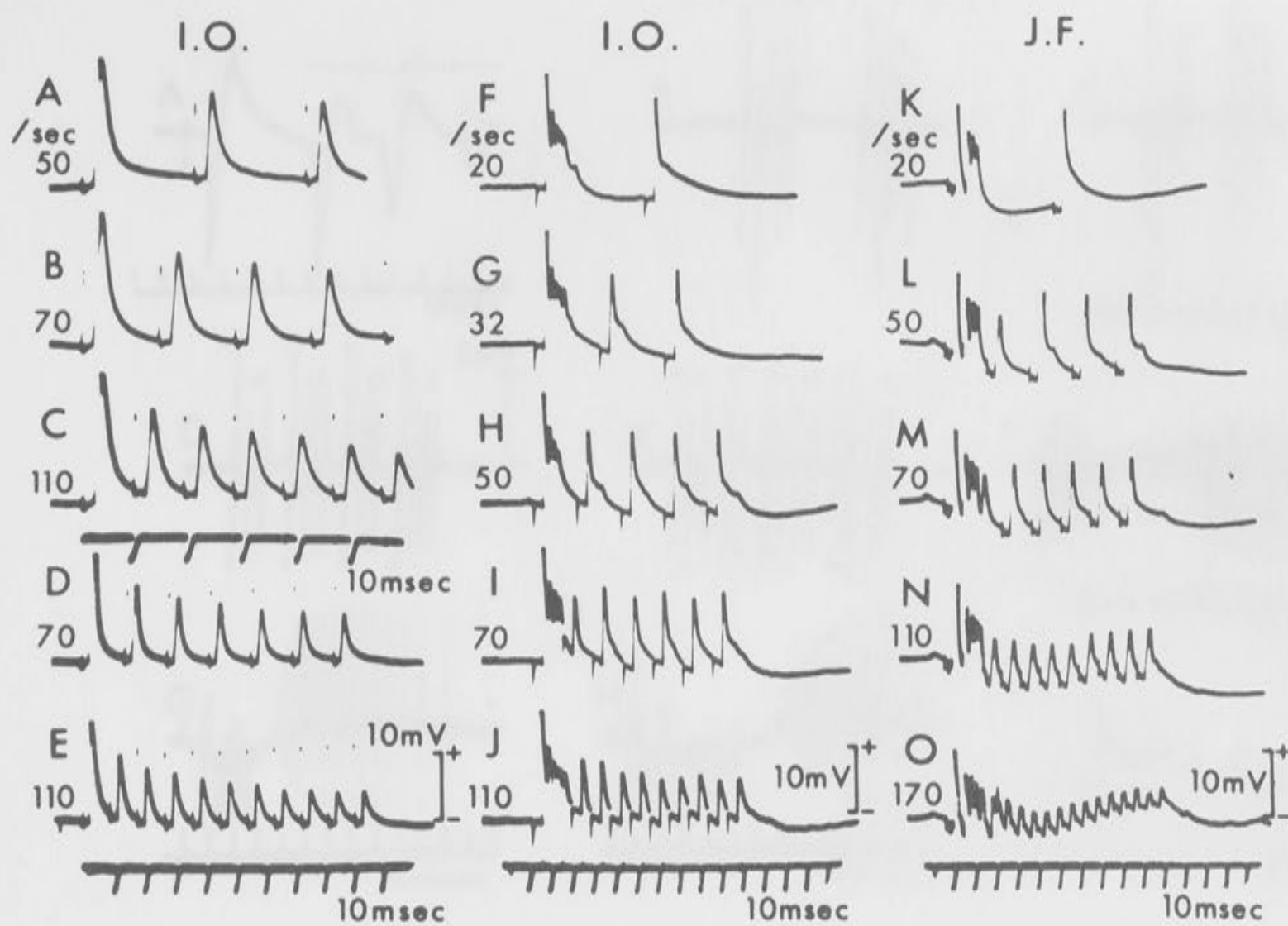


Fig. 22.

Intracellular records of the CF EPSP produced in Purkinje cells by repetitive I. O. and J. F. stimulation.

Series A-E and F-J show responses of two different Purkinje cells to I. O. stimulation, while K-O gives the responses of the same cell as in F-J but for J. F. stimulation. The stimulation frequency is noted at the left of each record as number of stimuli per second, and the time and voltage scales are given for each series.

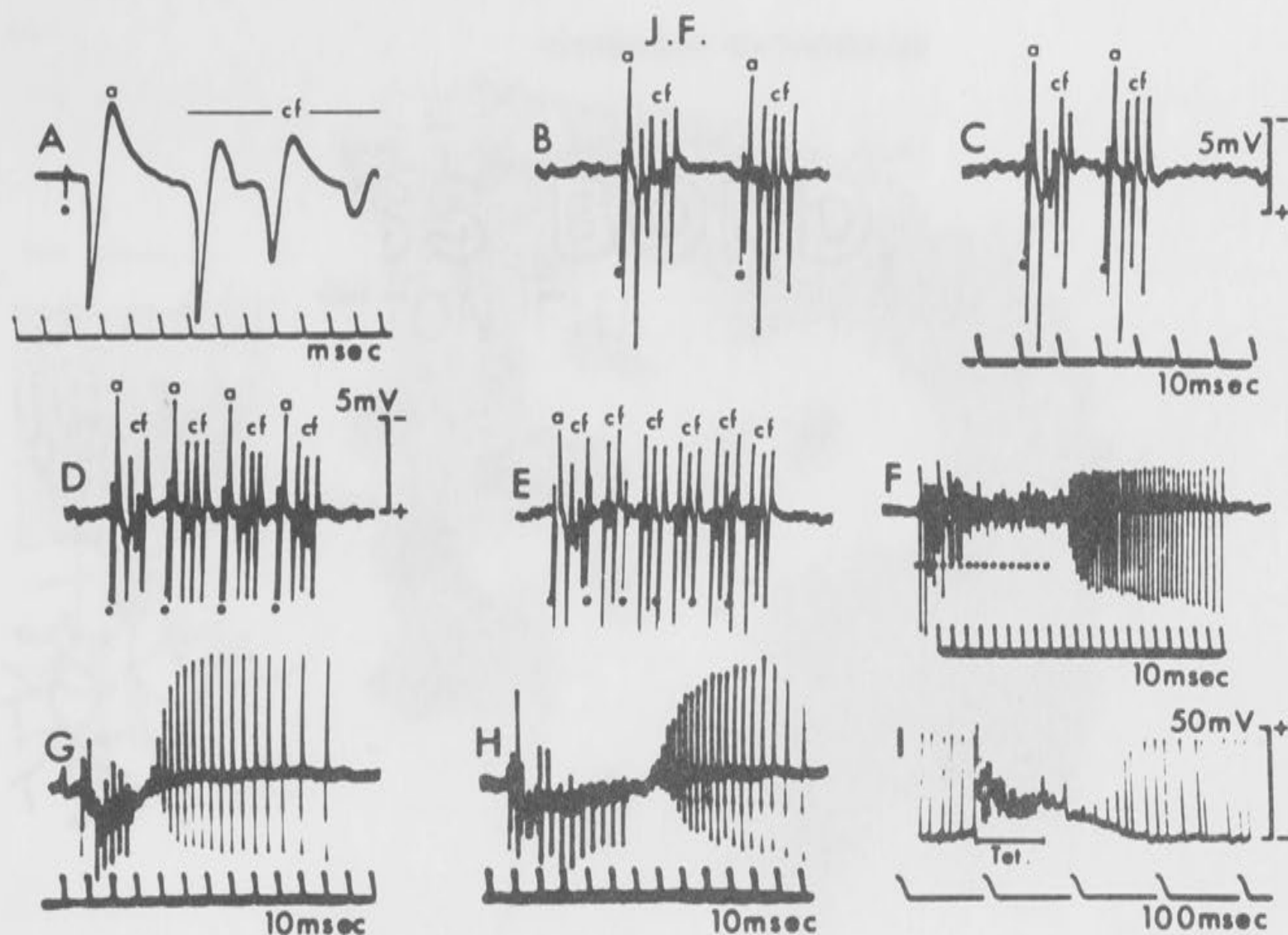


Fig. 23.

Extracellular and intracellular spike activity evoked in Purkinje cells by repetitive CF activation by tetanic J.F. stimulation.

In A, J.F. stimulation evoked a diphasic antidromic spike of the Purkinje cell (a), followed by the CF spike complex (cf).

In B-F the J.F. stimulation was repeated at different frequencies (see text).

In records B-D, every J.F. response consisted of the two components a and cf.

Record E shows that antidromic invasion failed after the first J.F. stimulation.

In record F a high frequency tetanus produced a failure of the large spike potentials after six stimulations, and shortly after the tetanus there was a prolonged after-discharge.

Records G-H illustrate similar responses evoked in another Purkinje cell.

In I, an intracellular record from a Purkinje cell shows the prolonged depolarization and spike inactivation produced by summation of the repetitively produced CF EPSPs by a tetanic J.F. stimulation for the duration of the horizontal line. The depolarization outlasts by 100 msec. the duration of the stimulus. The same time and potential scales obtain for records A-E and the potential scale also holds for F-H that are at slower time scales. There are separate scales for I. In B-F stimuli are indicated by dots.

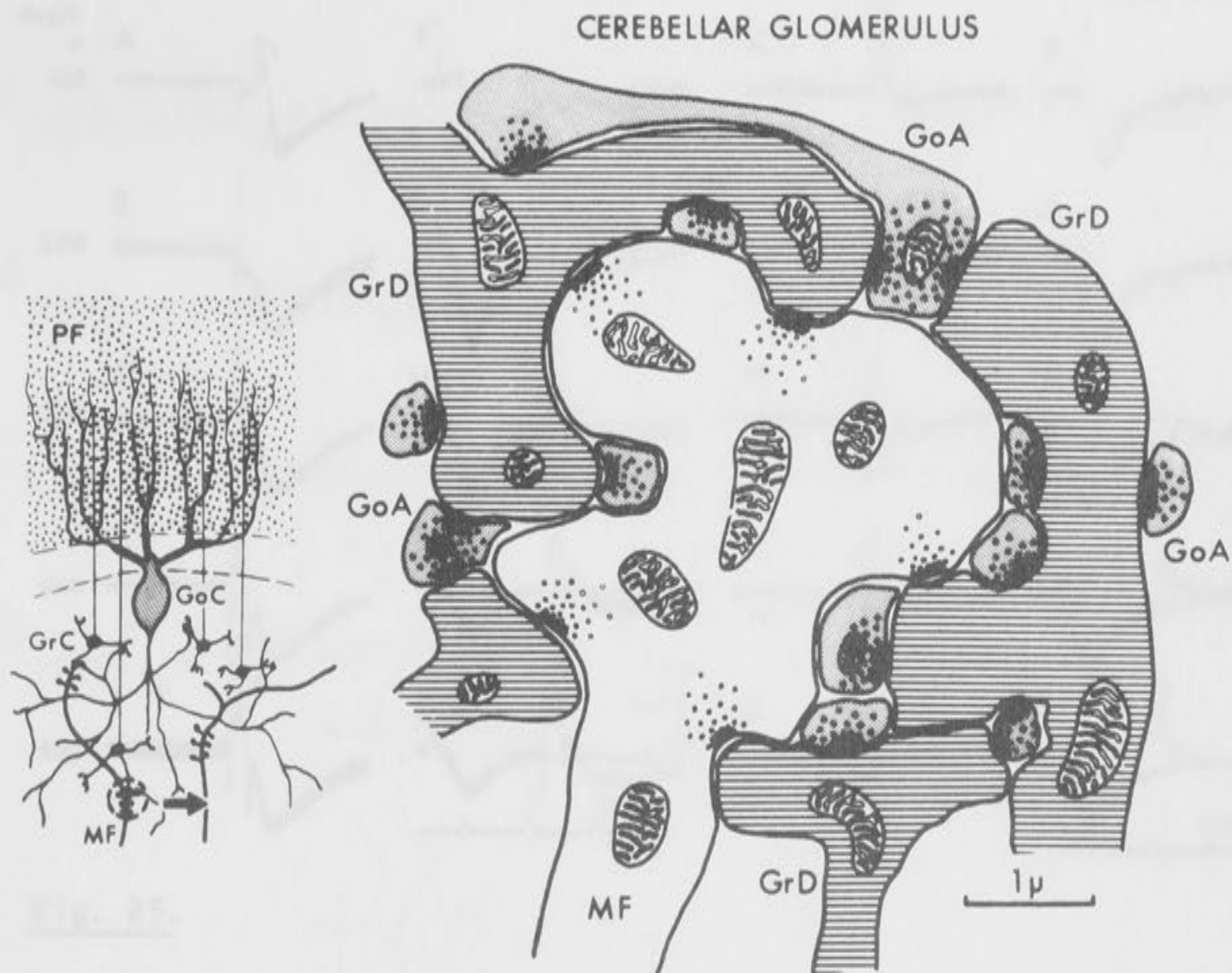


Fig. 24.

Diagrammatic representation of the cerebellar glomerulus. At the left, a Golgi cell (Go C) is drawn in the granular layer; its dendrites run upwards into the molecular layer where they enter into synaptic contact with the parallel fibres (PF). The axons of the Golgi cells have a complex relation with the synapse between the terminal sac of a mossy fibre (MF) and the dendrites of the granule cells (GrC). At the right is a schematic drawing (modified from Szentagothai, 1963) of the electron microscopic structure of the glomerulus (encircled area at the left). The mossy fibre terminal convolution, MF (white), enters into synaptic contact with the dendrites of granule cells, GrD (shaded by horizontal lines). The Golgi cell axon terminals, GoA, also form synapses with the dendrites of the granule cells.

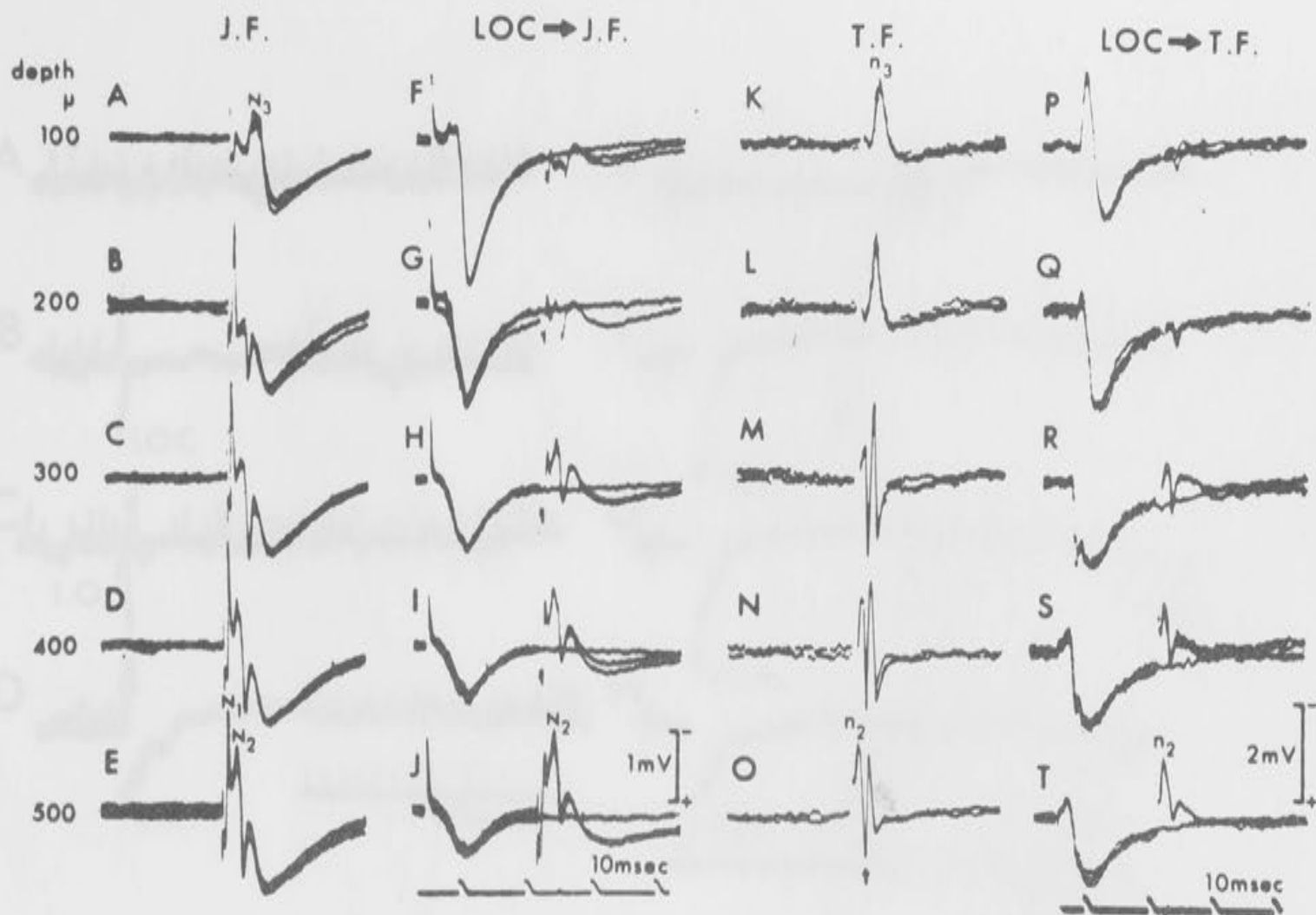


Fig. 25.

Field potentials evoked by the juxta-fastigial (J. F.) and trans-folial (T. F.) stimulation at different depths in the cerebellar cortex.

A-E: field potentials evoked by J. F. stimulation and recorded with a microelectrode at 100_μ steps, down to 500_μ depth. As indicated in E, the J. F. evoked field potential at the depth of more than 200_μ consists of two negative components N₁ and N₂, the former representing the spikes of the Purkinje cells and of the mossy and climbing fibres, the latter corresponding to the activity of the mossy fibre-granule cell relay (M. G. R.). At 100_μ depth (A) a third negativity N₃ is seen, and is considered to be due to the activation of parallel fibres and their excitatory actions on Purkinje cells.

In K-O, similar field potentials were evoked by T. F. stimulation. At 300 to 500_μ depth (Record M-O) the n₂ wave is split by a sharp positivity (marked with an arrow in O) which is attributed to the activation of the ascending axons of granule cells (see text). As in record A, an n₃ wave was recorded at 100_μ and 200_μ depth (K and L).

In records F-J and P-T, J. F. and T. F. stimulations were preceded by a local stimulation (LOC), which produced a large depression of the N₃ and n₃ waves without affecting much the N₂ or n₂ components, but the subsequent positivity was greatly depressed. Note different voltage calibrations for J. F. and T. F. series, but the same for both sets.

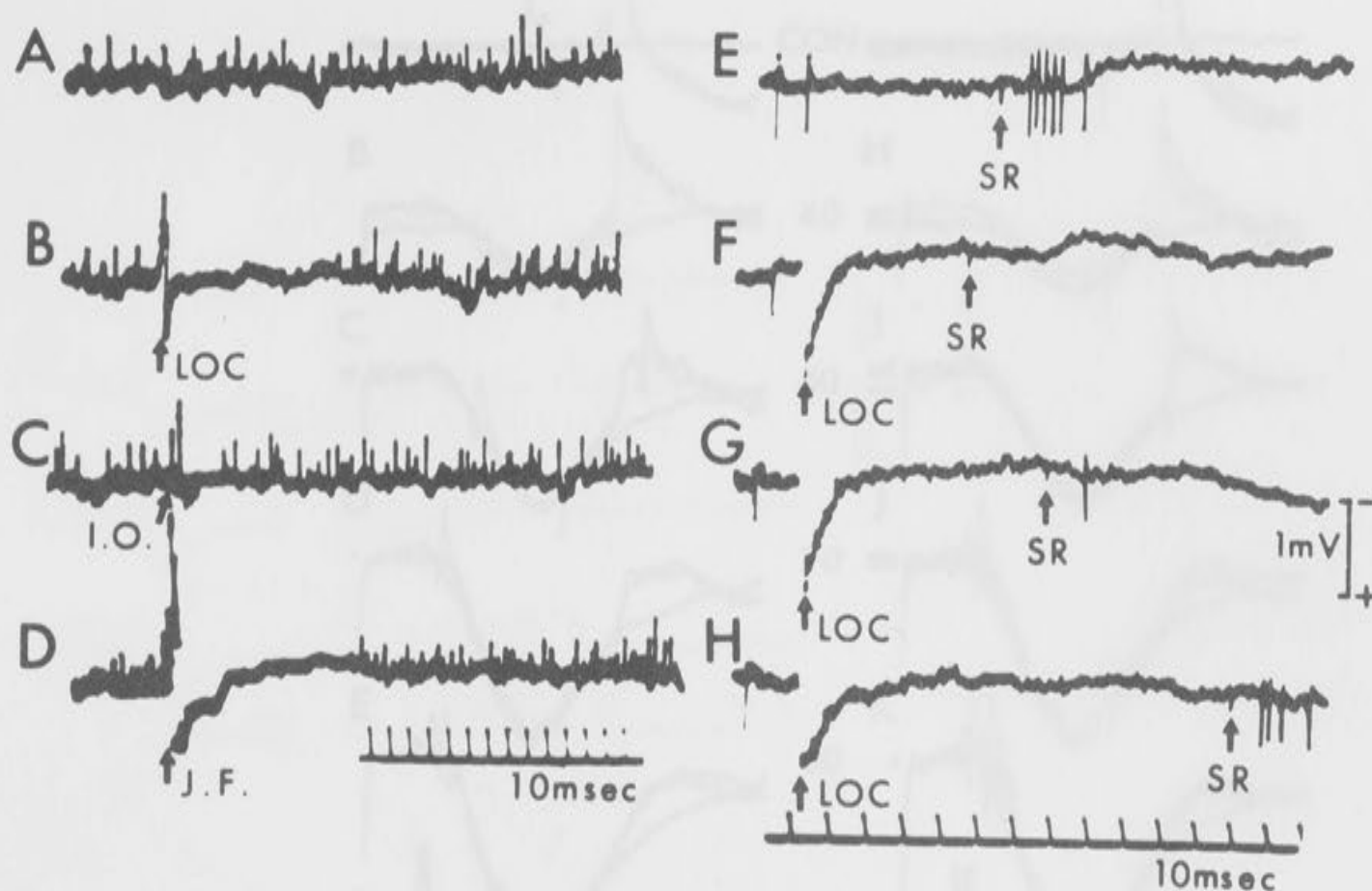


Fig. 26.

Inhibition of repetitive firing of impulses by granule cells in response to LOC, J. F. and inferior olive (I. O.) stimulation.

Spontaneous activity of several granule cells recorded at 600_u depth (control in A) was inhibited, as seen in B, C and D, by LOC, I. O. and J. F. stimulus respectively. Note the complete removal of the background spontaneous discharge following each stimulus. In E a single granule cell was fired repetitively by a single stimulus to the superficial radial nerve (SR). In F-H this response was inhibited by a conditioning LOC stimulus which preceded the SR stimulus at increasing intervals. Note different time scales for records A-D and E-H. Stimulus artifacts are marked with arrows.

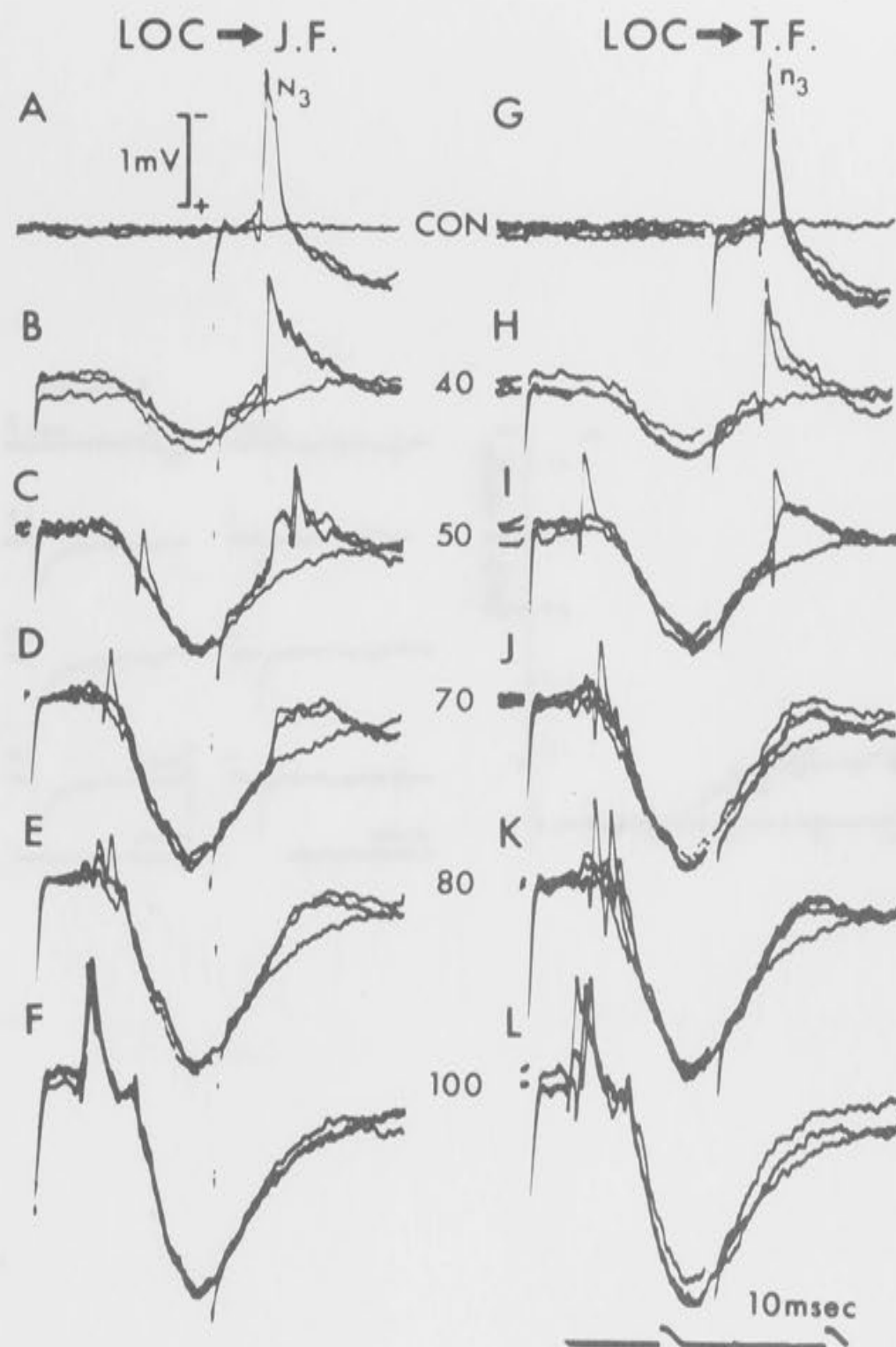


Fig. 27.

Field potentials evoked by J.F. and T.F. stimulation in the molecular layer (depth 200 μ), and their depression by a preceding LOC stimulation of increasing strengths. In the control responses A and G a small and brief initial positivity was followed by a large negative potential, N_3 and n_3 respectively, and a later large and slow positive wave. In B-F and H-L, N_3 and n_3 were progressively more depressed by increasing strength of LOC stimulation, the depression being virtually total in F and L. All-or-nothing Purkinje cell spikes are seen riding on the remaining negative potential in B, C, H and I. Note the early negative wave with superimposed Purkinje cell spikes evoked by the strong LOC stimulation in records F and L. Time and potential scales are the same for all records.



Fig. 28.

Time course of the Golgi cell inhibition of the T.F.-evoked field potential at the molecular layer, by a preceding LOC stimulation.

A, E and I are controls of the T.F. field potentials evoked at 140_{μ} depth at different sweep speeds. In B-D, F-H and J-L, T.F. stimulations were delivered at increasing intervals after the conditioning LOC stimulation, there being still a small inhibition in L at 560 msec after the LOC stimulation (note the different time scales). M is a plot of the time course of the Golgi cell inhibition. The amplitude of the T.F. response is plotted in the ordinates against the intervals between the LOC and the T.F. stimulation in the logarithmically scaled abscissae.

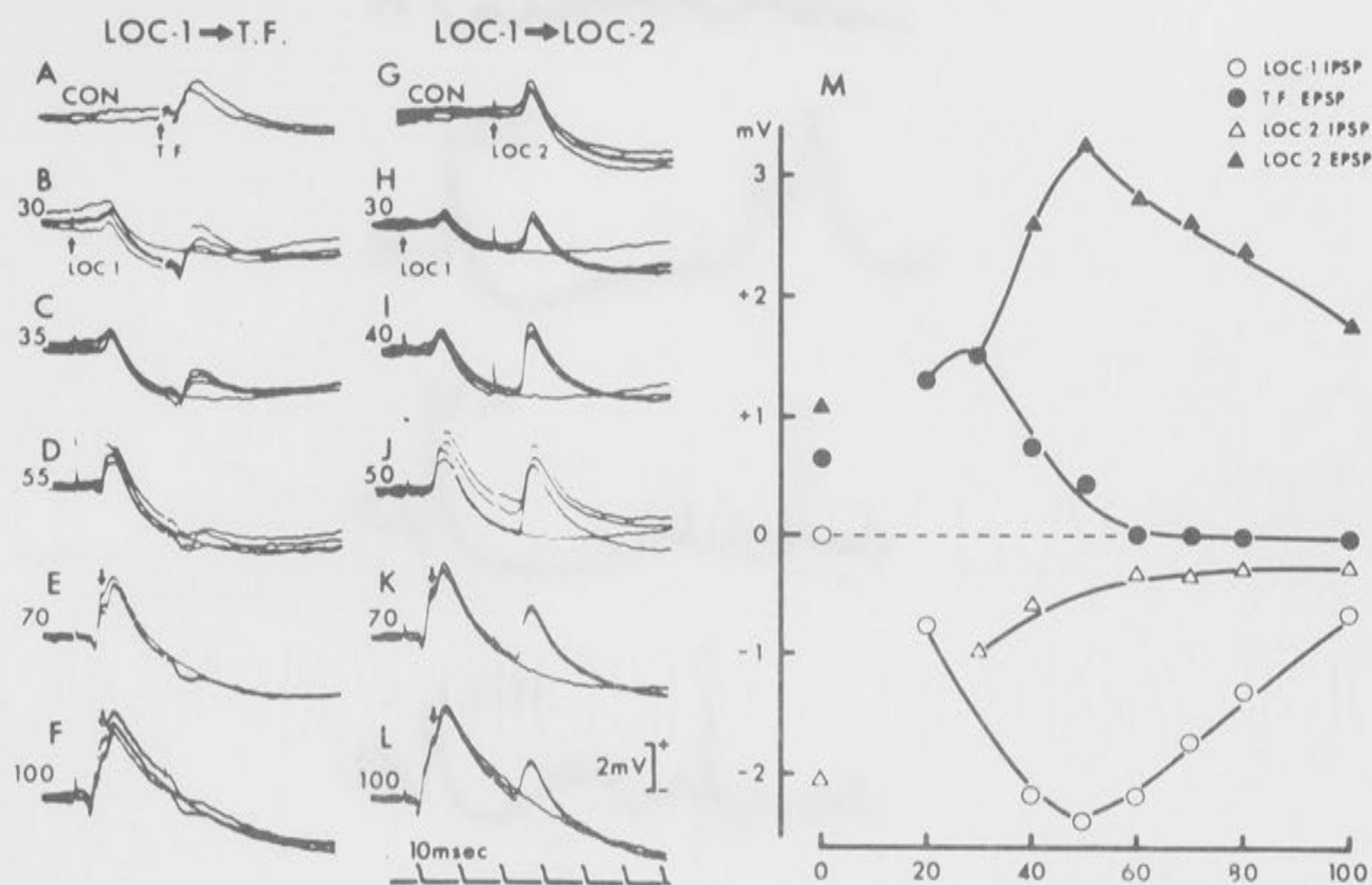


Fig. 29.

Differences between the effects of a preceding LOC stimulation on the EPSPs in a Purkinje cell evoked by T.F. and by LOC stimulation. The EPSP evoked by T.F. stimulation (control in A) was depressed by a preceding LOC stimulation of increasing strengths (B-F). In G the EPSP was evoked in the same cell by a LOC-2 stimulus and was not depressed by the conditioning LOC-1 stimulus but enhanced. Stimulus strengths of the LOC-1 are marked in arbitrary units at the left of the records. Note that, in records E, F and K, L strong LOC-1 stimulation produced an early EPSP marked with an arrow. All records at the same time and voltage scale. In M are plotted measurements (note the identifying symbols) from a series similar to that of A-L and from the same Purkinje cell, the ordinates being potentials and the abscissae the LOC-1 stimulus strengths in arbitrary units. The points for the T.F. and LOC-2 EPSP were measured relative to the baseline formed by the LOC-1 response.

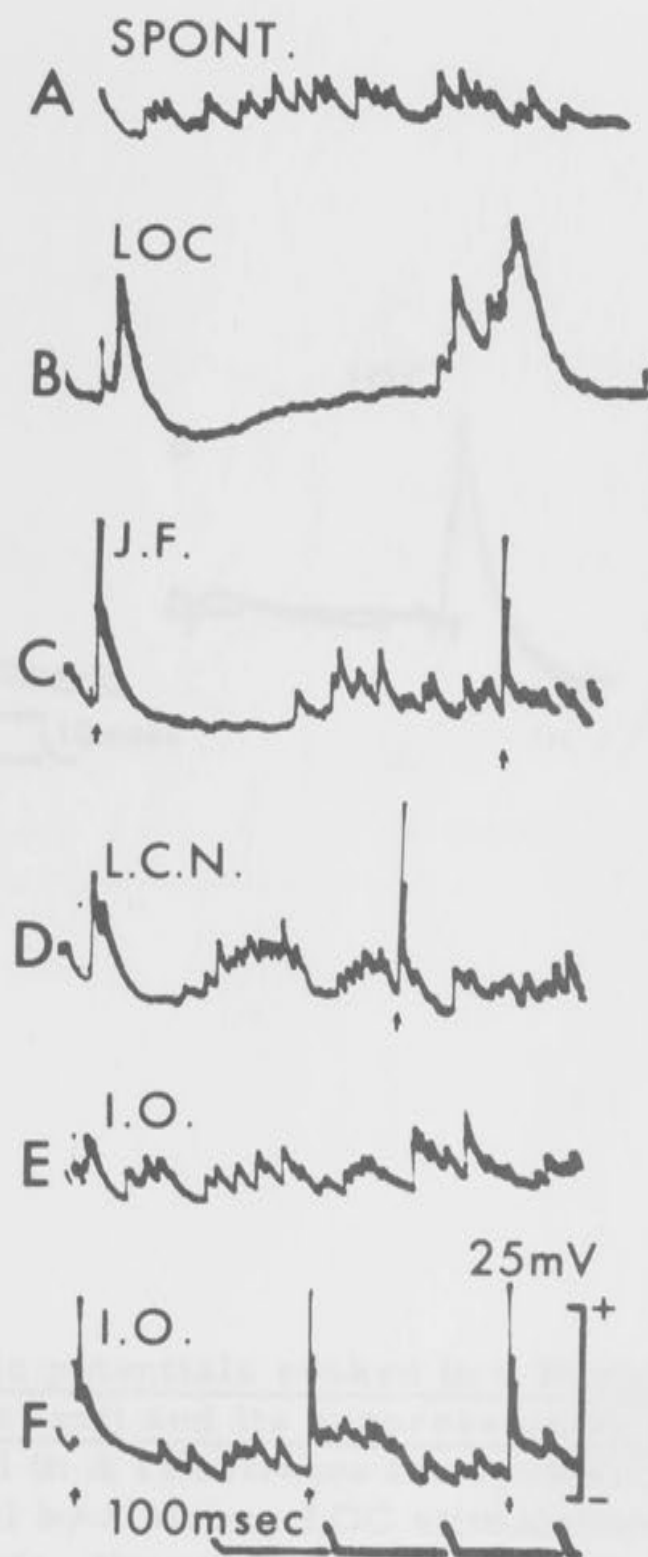


Fig. 30.

Background synaptic bombardment recorded intracellularly from a Purkinje cell and its suppression by the Golgi cell inhibition.

Record A illustrates the background inhibitory synaptic barrage invested by Cl^- injection into the cell. In B, this background bombardment was suppressed for about 140 msec by a LOC stimulation. A similar suppression was found in records C and D with a J. F. and a L. C. N. stimulation respectively. In E, a weak I. O. stimulation produced a small depression of the background activity and in F a stronger I. O. stimulation increased the duration of the background depression. Note the spontaneous climbing fibre EPSP marked with arrow in records B, C, D and F. Time scale 100 msec, voltage calibration 25 mV.

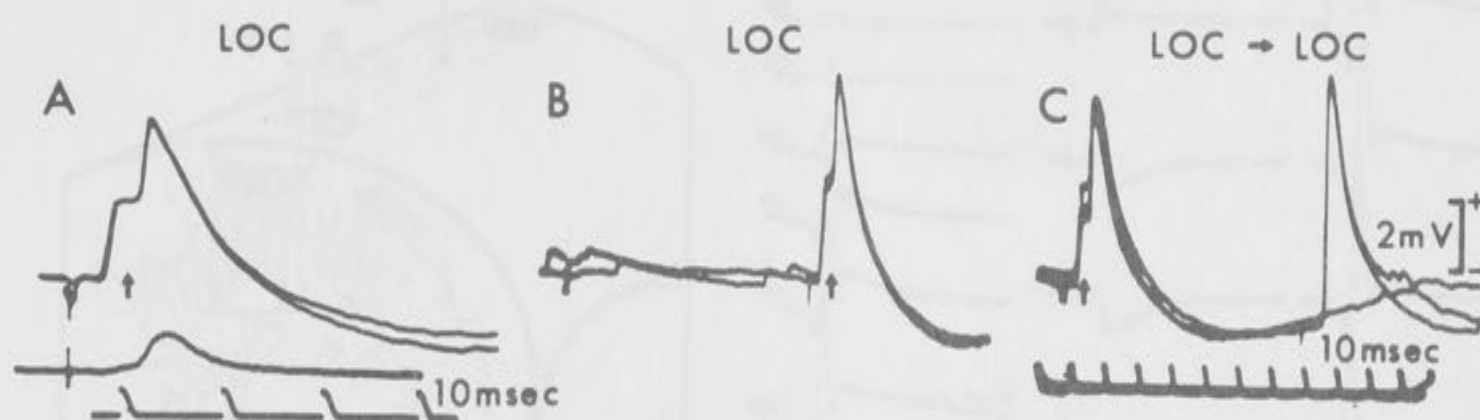


Fig. 31.

Synaptic potentials evoked in a Purkinje cell by the mossy fibre reflex (see text) and its suppression by Golgi cell inhibition. An upper record in A illustrates the synaptic potentials evoked in the Purkinje cell by a strong LOC stimulation. The EPSP consists of two components, the early one (marked in all records with an arrow) being produced by the direct activation of mossy fibres due to the LOC stimulation, the second one by the direct activation of parallel fibres. The lower record illustrates the extracellular field potential. In B this synaptic potential sequence is illustrated at a slower sweep speed and serves as a control for record C. In C a preceding LOC stimulation suppressed completely the early mossy fibre-evoked EPSP, but did not reduce the amplitude of the EPSP produced by the direct action of the parallel fibres. Time scales 10 msec, voltage calibration 2 mV.

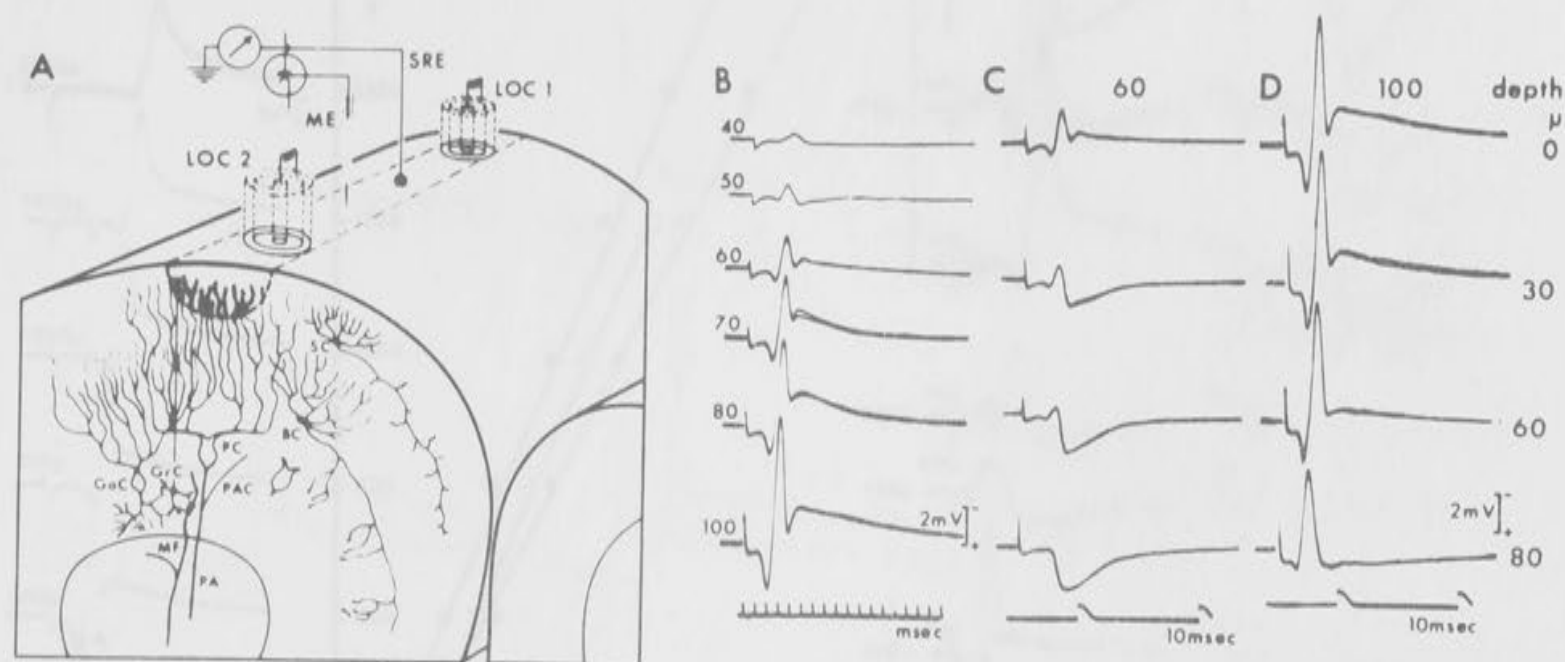


Fig. 32.

A. Diagram of section of a folium of the cerebellar cortex showing the location of the concentric stimulating electrodes on the surface (the local or LOC electrodes). Also shown is the surface recording electrode (SRE) and the microelectrode (ME) in position for penetration. Purkinje cell (PC) shown with axon (PA) and axon collateral (PAC). Also shown are superficial stellate (SC), basket (BC) and Golgi (GoC) and granule (GrC) cells. The broken line shows the boundaries of a beam of parallel fibres excited by a weak LOC stimulus.

B. Extracellular potentials evoked by graded LOC stimulations at the indicated strengths in arbitrary units and recorded by a microelectrode that had just penetrated into the molecular layer.

C. Potentials produced at strength 60 as in B, but recorded at the indicated depths in D below the surface of the molecular layer.

D. As in C, but for strength 100.

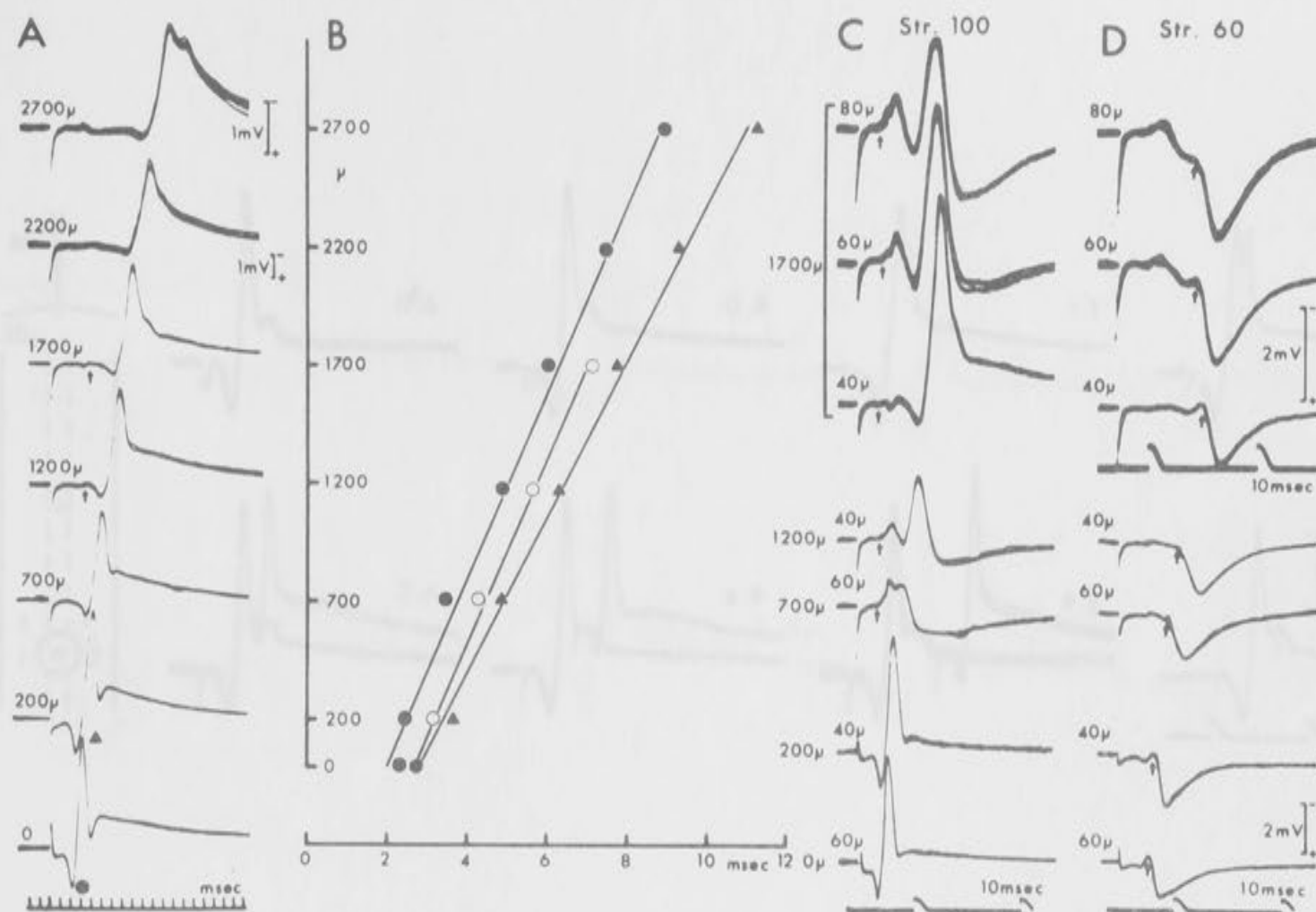


Fig. 33.

Extracellular potentials produced by LOC stimulation and recorded in line with the beam of excited parallel fibres and at various distances along the folium from the LOC electrode.

A. LOC stimulus strength 100 and recording just below surface at the indicated distances from the recording position closest to the LOC electrode. All records at same amplification except that at 2700 μ .

B. Plotting of conduction distances against the latencies measured for peaks of initial positivity and negativity as indicated in A, and also for onsets of slow positive wave as indicated in D. C and D are potentials evoked by LOC stimuli of strengths 100 and 60 respectively and recorded at the indicated depths below the surface. The upper three traces were at 1700 μ position (see A), and the lower four in each column at 1200, 700, 200 and 0 positions as indicated. The potential scales for the upper three and the lower four records are different.



Fig. 34.

Extracellular potentials evoked by double stimulation through the same LOC electrode. Diagram shows folium with LOC electrode in plan and with the excited beam of parallel fibres shaded. Microelectrode (ME, 30 μ depth) penetration was at the cross. CON shows control response to second LOC stimulation alone, and with arrow indicating a possible Purkinje spike potential. Stimulus intervals indicated on each record in msec, and at 3.4, 4.9 and 9.2 msec the double response was superimposed on the response to the first LOC stimulus alone.

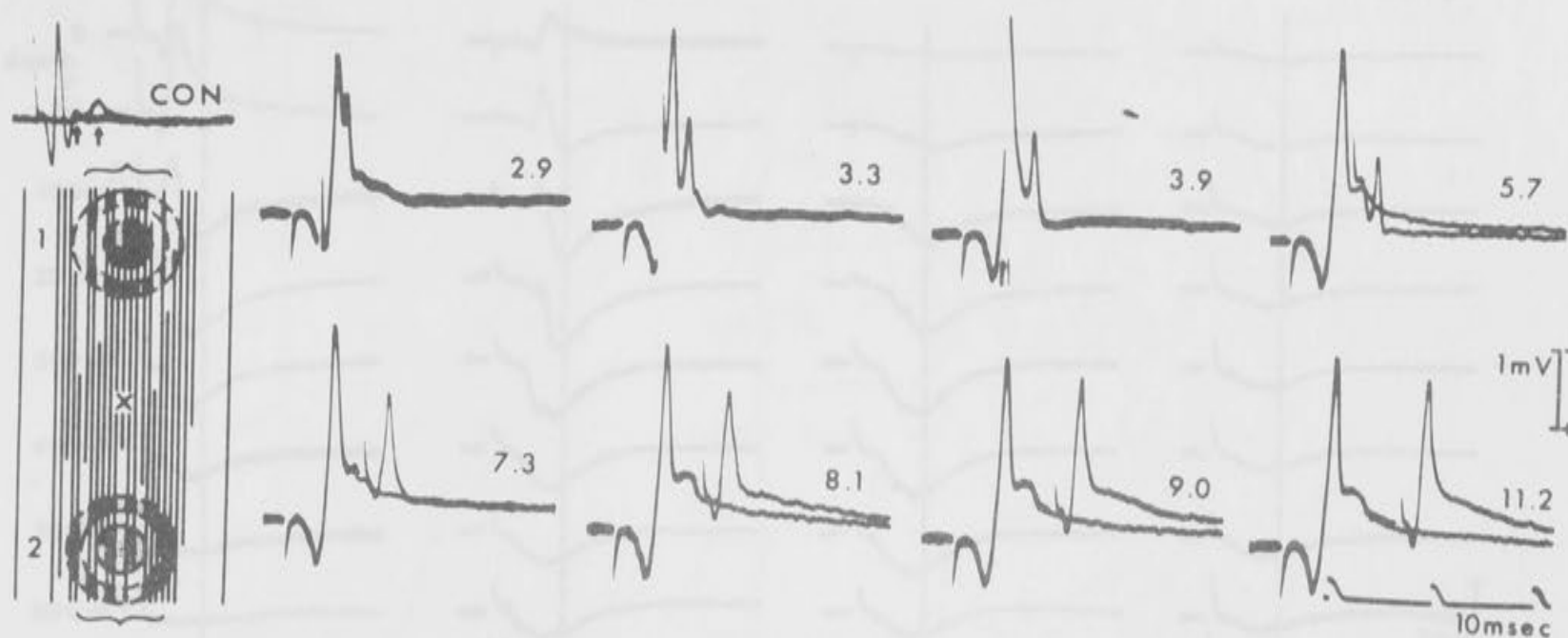


Fig. 35.

Interaction by collision of two parallel fibre volleys.

As shown in diagrammatic plan two LOC electrodes (1 and 2) were placed on a folium in accurate longitudinal alignment. The longitudinal lines show diagrammatically the parallel fibres that could be excited by one or both electrodes and recorded from by the microelectrode inserted at the cross. CON shows the control response to the second LOC stimulus alone superimposed on a base line and with arrows indicating possible Purkinje spike potentials. In all other traces the first LOC stimulus preceded the second at the indicated intervals in msec. There was superposition on the control response to the first LOC stimulus for intervals from 5.7 to 11.2 msec.

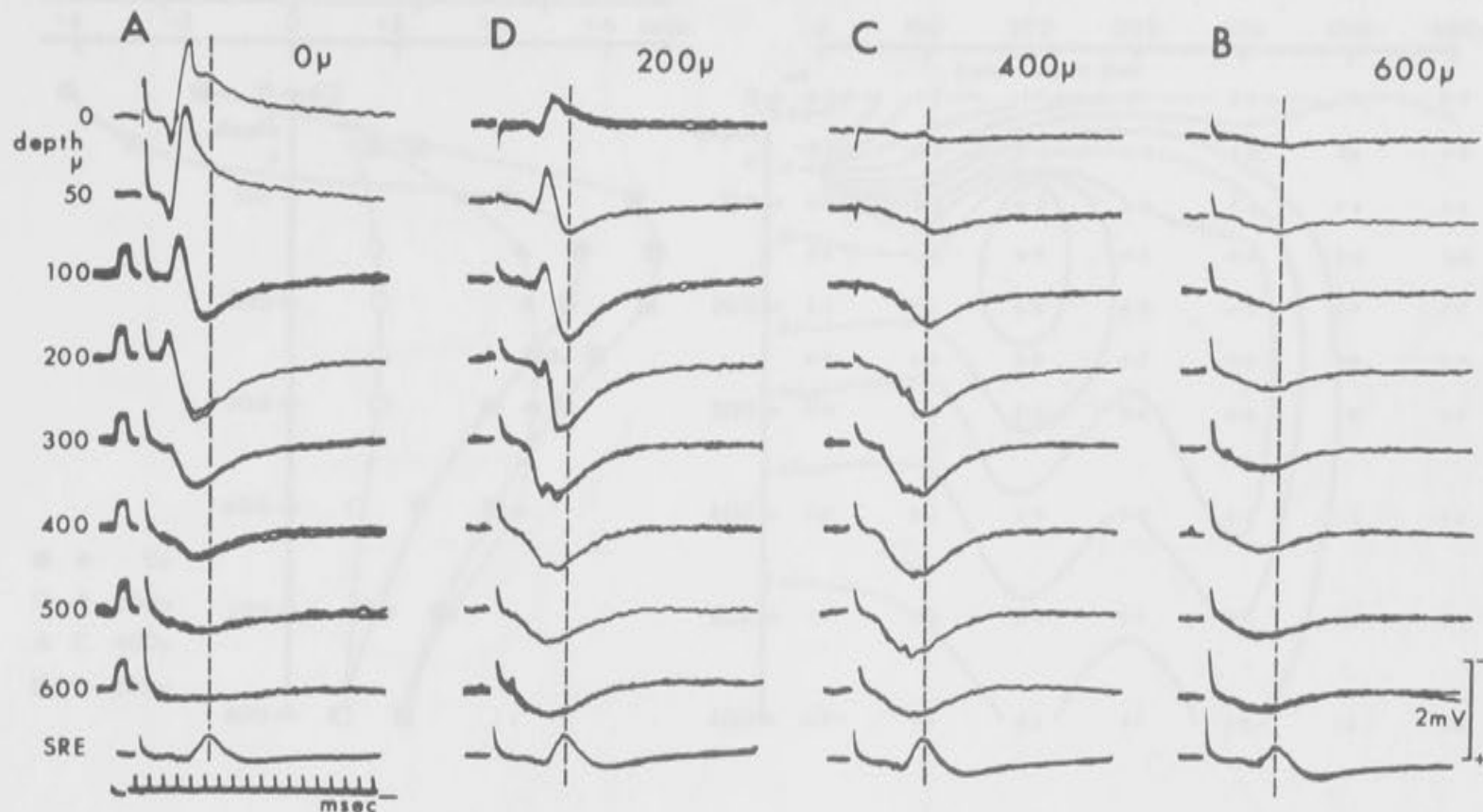


Fig. 36.

Field potentials produced in a cerebellar folium by a standard LOC stimulus in a chronically (22 days) deafferented cerebellum. The potentials recorded in four tracks are arranged in four columns at the indicated depth sequences, the lowest trace in each column being the monitored response of the parallel fibre volley recorded by the SRE (Fig. 32) at least 1 mm further from the LOC electrode than the microelectrode. The track recordings were made in the sequence A, B, C, D as indicated, A being accurately on the beam of excited parallel fibres, B 600 μ lateral thereto and C, D at lateralities of 400 and 200 μ . All traces were at the same sweep speed and amplification. Perpendicular broken lines indicate point of measurement of all traces at 5.3 msec latency.

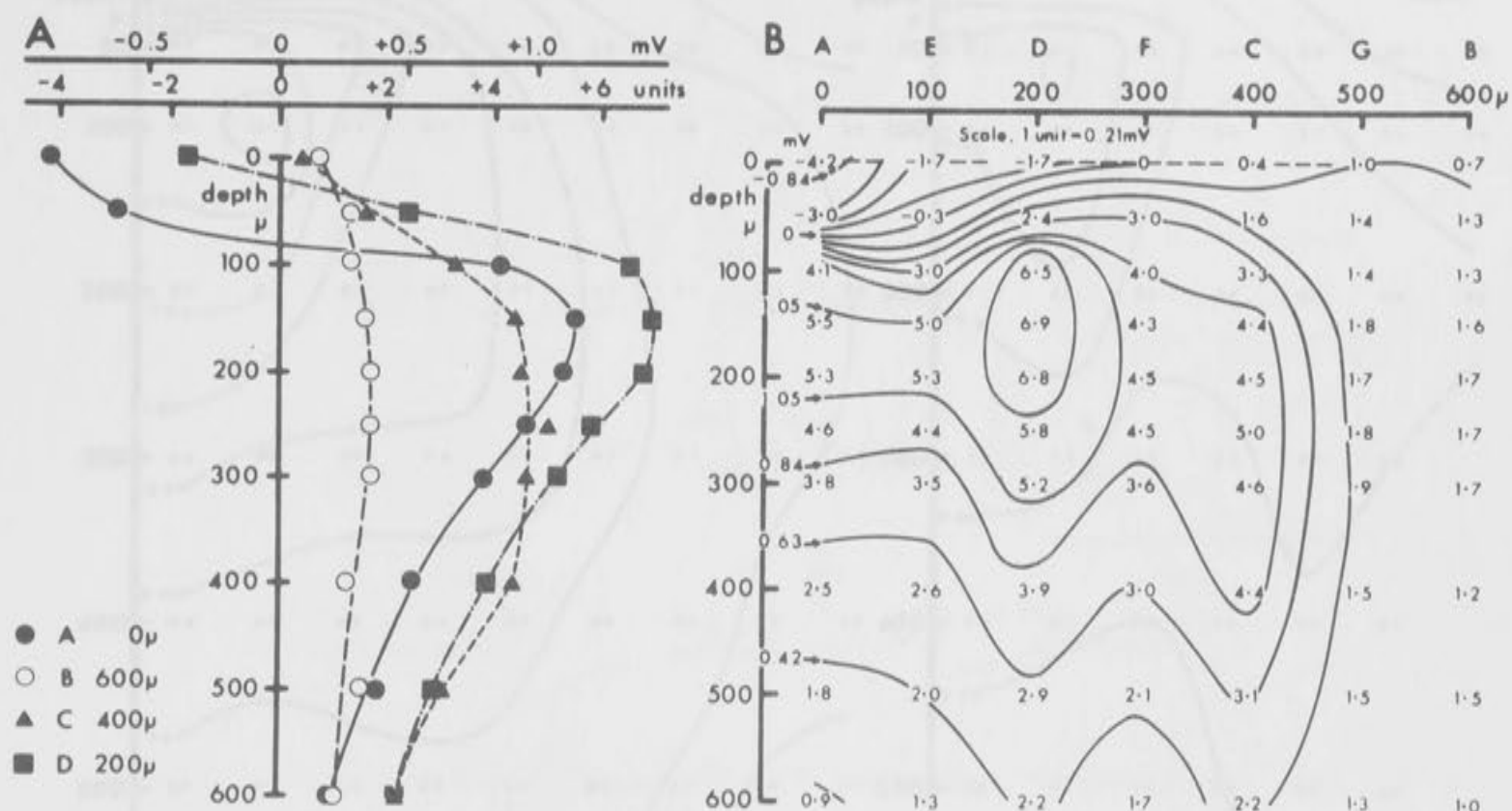


Fig. 37.

Plotting of field potentials for the series illustrated in Fig. 36. A is a plotting at the various depths of the potential measurements (in arbitrary units and mV) at 5.3 msec latency for the records of Fig. 36. The tracks A, B, C and D are indicated by the symbols. B. On the grid of transverse and depth measurements for the sites of recording in the seven tracks there are indicated the potential measurements in arbitrary units at 5.3 msec latency for the series of Fig. 36, and also for the three later tracks E, F and G. Approximately potential contour lines are drawn at the potentials in mV indicated by arrows. There is a lateral and deep spread of the positive potential field in track C at 400_u lateral to the centre of the beam of excited parallel fibres (track A).

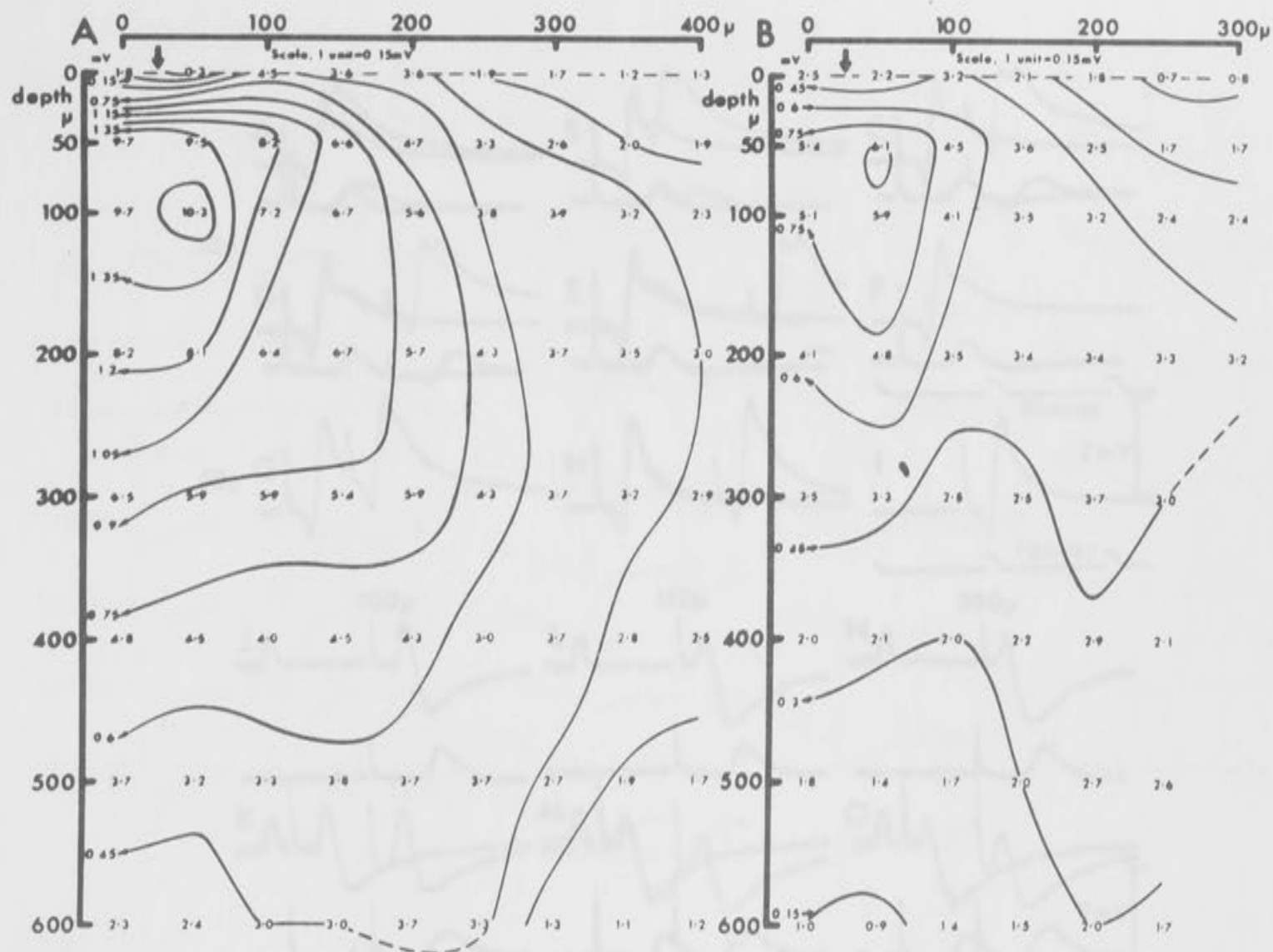


Fig. 38.

Contour plottings of field potentials generated by standard LOC volleys in a normal cerebellum and recorded transversely to the excited beam of parallel fibres.

A. Potential measurements are made from a series like Fig. 36 at 7.5 msec latency and are indicated in arbitrary units at each point of the grid made by 9 tracks at 50_u spacing and from 600_u up to surface. Approximate contour lines are drawn at the potentials in mV indicated by arrows. The centre of the excited beam of parallel fibres was shown by the initial "spike" potentials to be at the arrow about midway between the two left tracks. The LOC stimulus for A was at strength 70 and evoked responses that at some recording sites were considerably complicated by mossy fibre stimulation.

B. The series at LOC stimulus strength 60 had very little mossy fibre complication and is similarly measured and plotted.

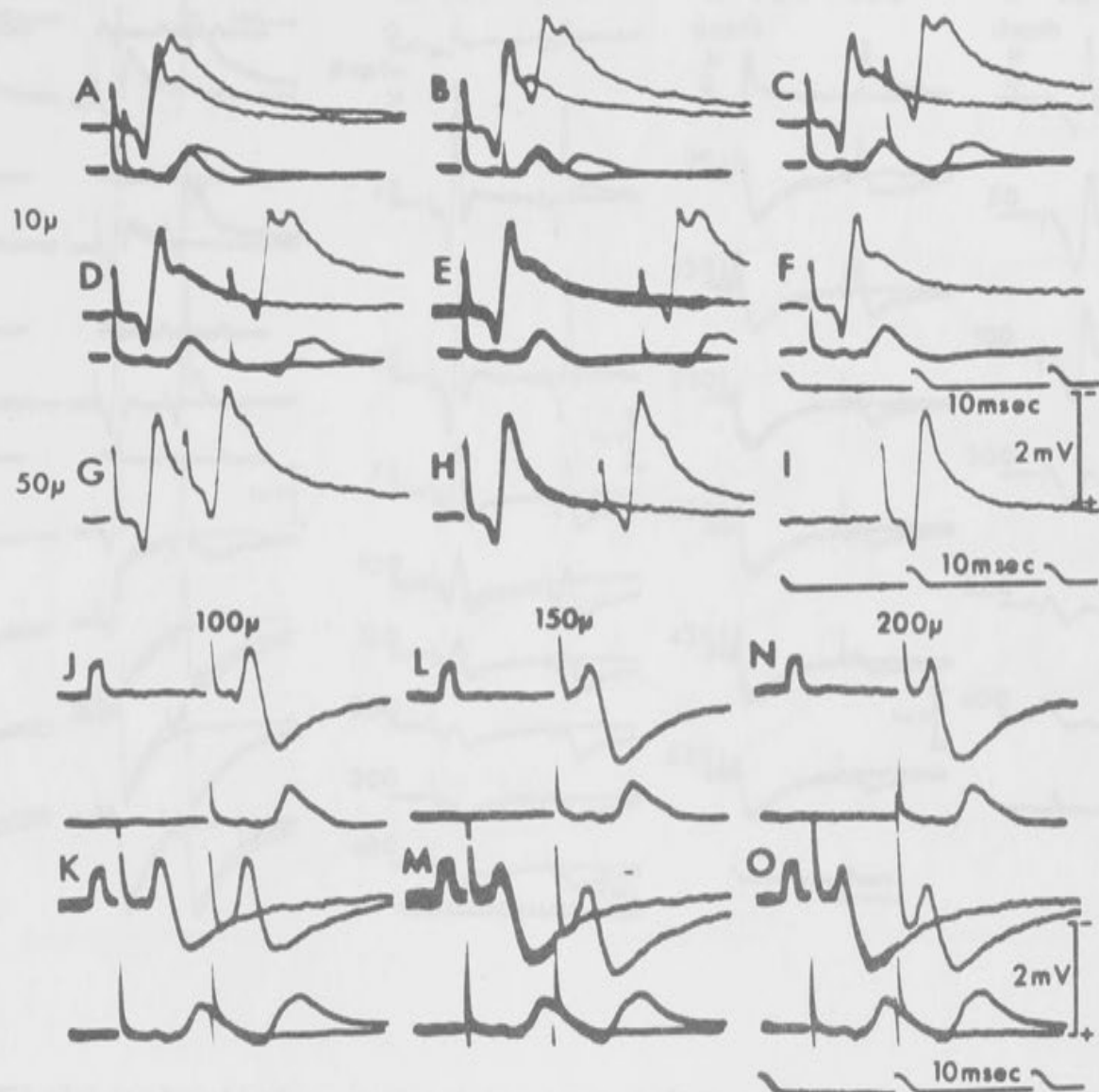


Fig. 39.

Extracellular responses evoked by two LOC stimuli in a chronically deafferented cerebellum (22 days). In A-F the upper traces were recorded by a microelectrode in the excited beam of parallel fibres and at a depth of 10μ , F giving the control response to the second LOC stimulus, and A-E to two LOC stimuli at various intervals, there being in each case a superimposed trace to the first LOC stimulus alone. Lower traces show responses of surface recording electrode. G-I is a similar series in the same track, but at 50μ depth, I being the control. J, K are also similar at one testing interval at 100μ depth, and L, M are at 150μ , and N, O at 200μ , all in the same track. Lower traces in J-O also show responses of surface recording electrode. Same strengths of LOC stimuli and same potential and time scales throughout.

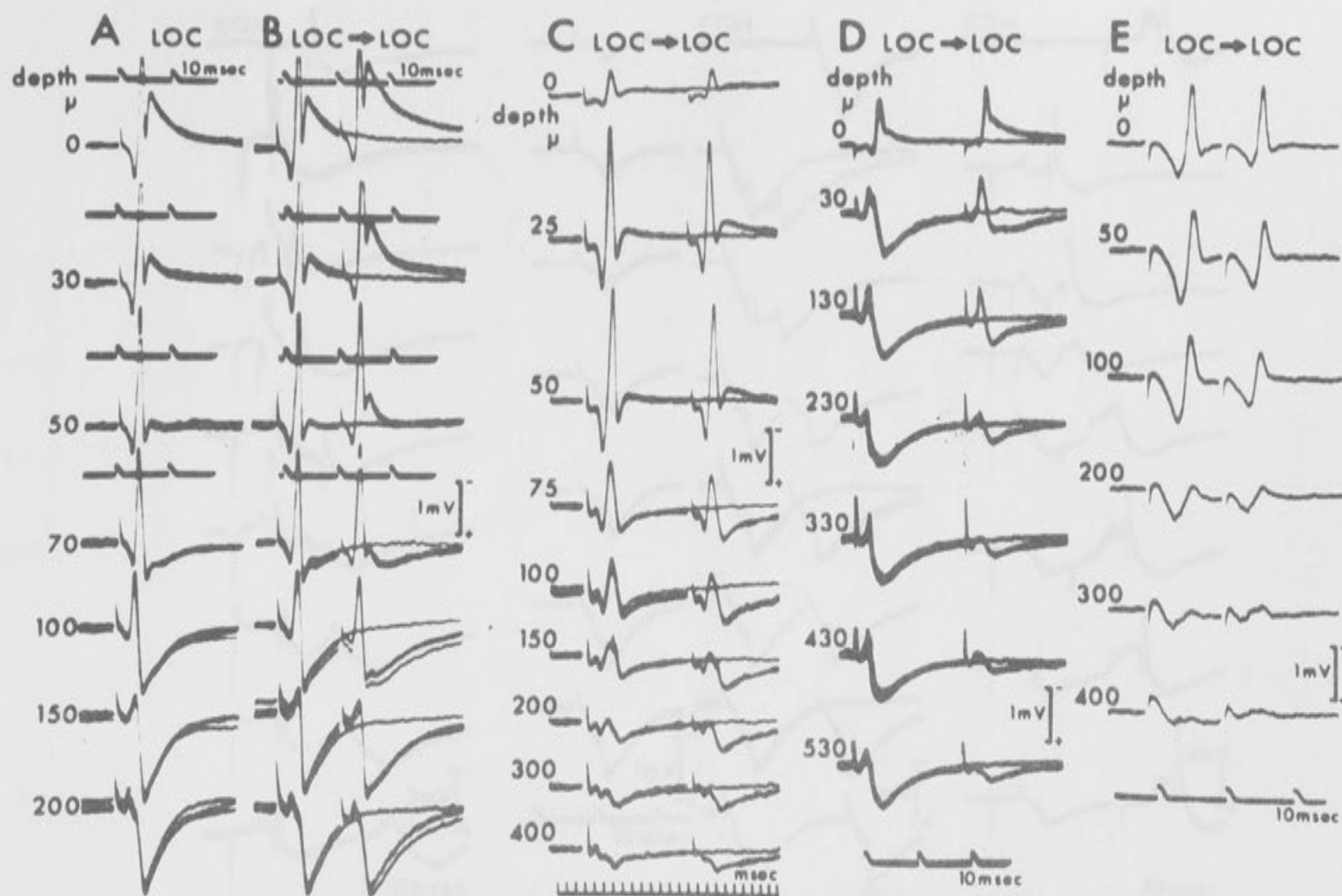


Fig. 40.

Field potentials evoked by two LOC stimuli at varying depths in the molecular layer.

A and B. Responses at the indicated depths in a chronically deafferented cerebellum to single and double stimuli of same strength and applied through the same LOC electrode. In B there were three traces to the double stimuli superimposed on control base line to the first LOC stimulus alone. The microelectrode was centrally placed on the beam of excited parallel fibres.

C. Another series also taken accurately in line with the excited beam of parallel fibres. The superimposed control response to the first LOC stimulus was identical with the control response to the second LOC stimulus that is not shown. The slow potential waves were abnormally small, probably because of the poor condition of the cortex.

D. Responses as in B, but for a normal cerebellum, the control being given by the first LOC response as in C.

E. A similar series but for a cortex just after failure of the circulation with a consequent survival of the parallel fibre volley uncomplicated by the slow potentials generated by synaptic activity. The series was taken in the order 0, 100, 200, 300, 400_μ, the return to the 50_μ depth showing that there was little deterioration of the parallel fibre response during the series in depth.

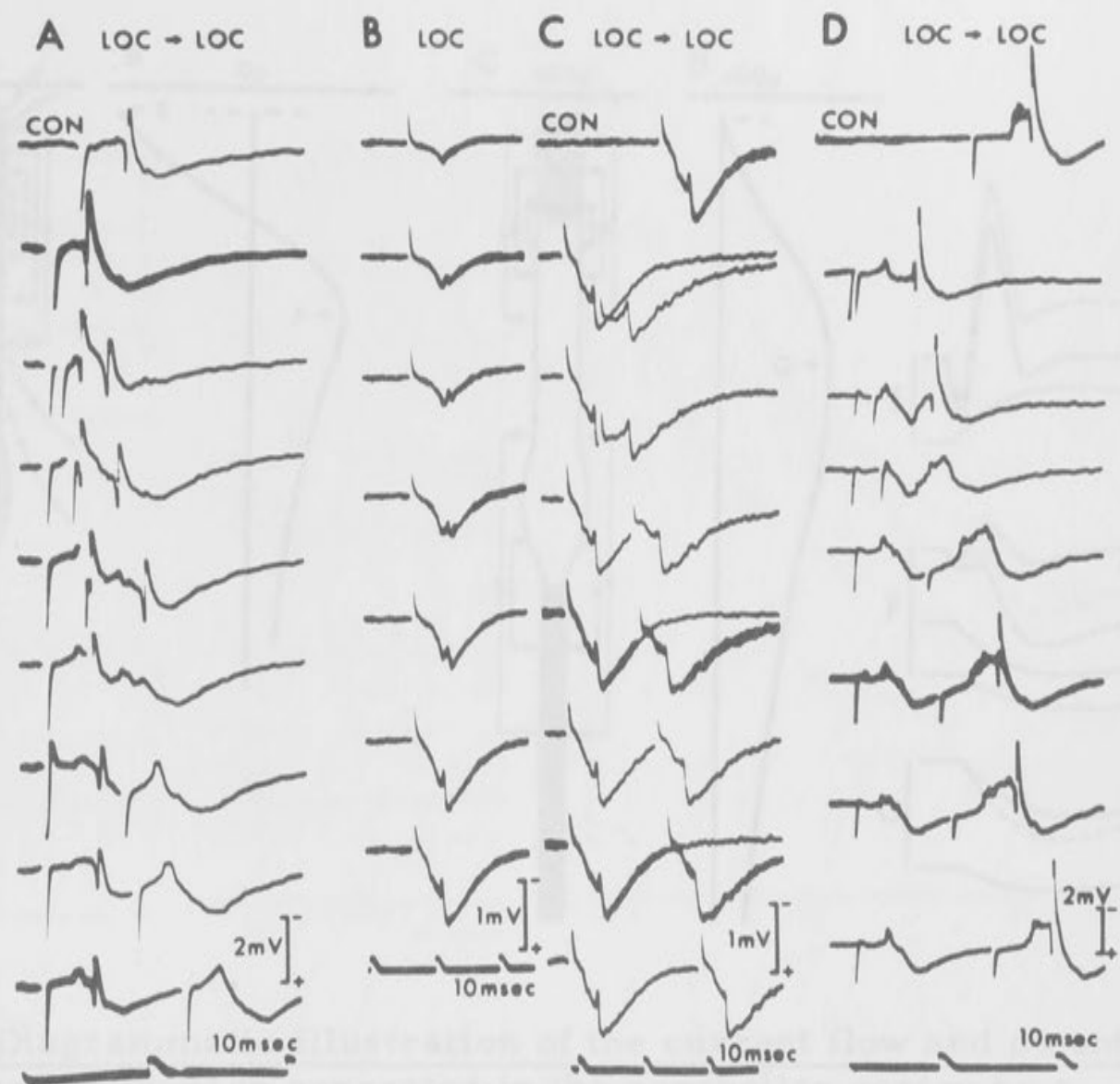


Fig. 41.

Extracellular records of spike potentials of Purkinje cells evoked by LOC stimuli.

A. The upper trace shows control response to second LOC stimulus at 250_{μ} depth. Below are the responses to two similar LOC stimuli at progressively longer intervals. The second stimulus failed to evoke a spike at the shortest interval (0.5 msec), but was successful at 0.9 to 2.8 msec.

B and C. Responses from a chronically deafferent cerebellum (9 days) at a depth of 250_{μ} to single LOC stimuli of progressively increasing strength (B), and (C), to two LOC stimuli at the strongest strength of series B at various intervals. Control of response to second LOC stimulus in upper record. Superimposition of response to first LOC stimulus alone in three records.

D. Responses of a normal cerebellum to stimuli applied through different LOC electrodes the recording depth being 200_{μ} . The second not conditioned LOC stimulus always evoked a single spike potential while the first always failed.

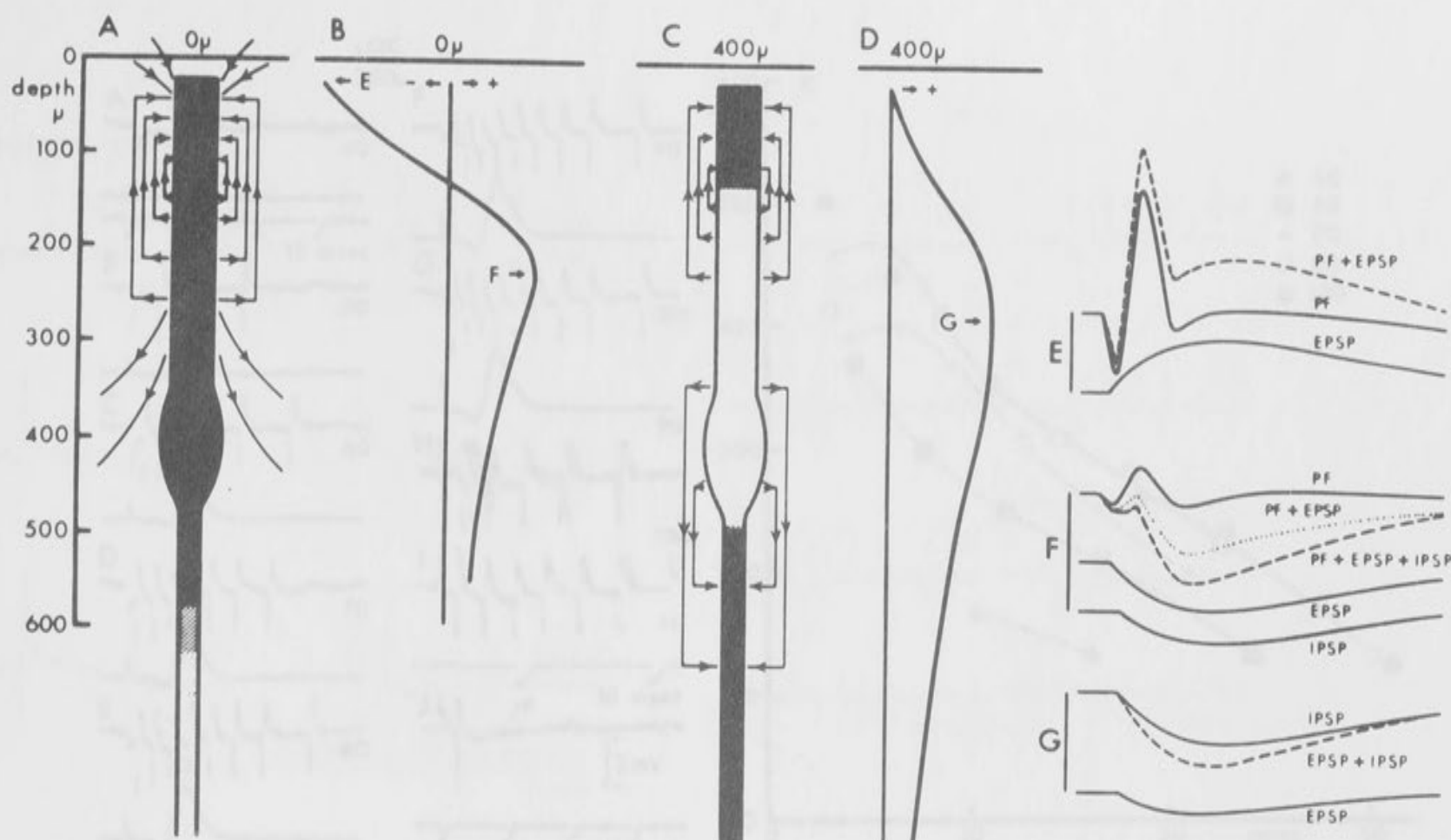


Fig. 42.

Diagrammatic illustration of the current flow and potential profiles and responses generated in the cerebellar cortex by a parallel fibre volley.

A. Schematic illustration of a Purkinje cell subjected to synaptic excitatory action by a parallel fibre volley to a depth of about 130_{μ} . The actively depolarized dendrites are shaded very darkly and the graded lighter shading shows the electrotonic spread of this depolarization produced by the indicated flow of currents.

B shows the potential profile produced by this current flow (see text).

C. A similar diagram to A, but for the inhibitory action of stellate and basket cells on a Purkinje cell 400_{μ} lateral to the centre of the beam of excited parallel fibres. The actively hyperpolarized area is very lightly shaded according to the same convention as in A.

D shows the potential profile produced by this focus of active hyperpolarization (see text).

In E the broken line shows the potential observed at the depth of E in profile B, the component potentials due to parallel fibre impulses and the EPSP being shown by continuous lines. Similarly in F and G the broken lines give the actually observed potential at points F and G in profiles B and D respectively, the various components being indicated by the labelled curves. Further description in text.

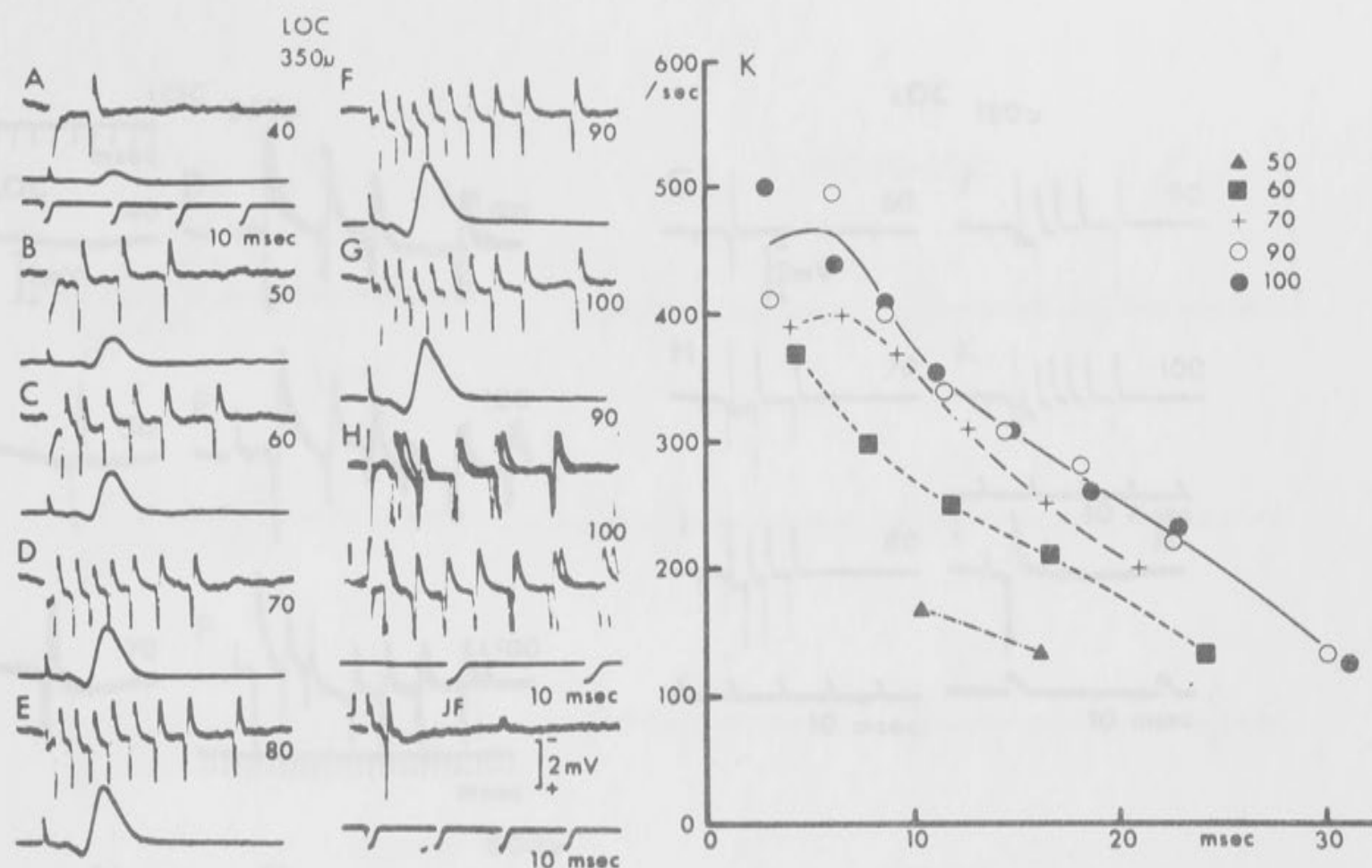


Fig. 43.

Extracellular recording of responses of presumed inhibitory interneurone at a depth of 350μ . A volley of impulses was generated in the parallel fibres by a stimulus of progressively increasing strength (given in arbitrary units) that was applied through a surface electrode (LOC) within 1 mm of the recording microelectrode. The lower trace of each record shows the mass spike potential produced by this parallel fibre volley and recorded by a surface electrode about 2 mm from the stimulating electrode, i. e. more than 1 mm beyond the recording electrode. All electrodes were placed in accurate alignment along the length of the folium. The superimposed traces of H and I were similarly produced in the same interneurone but at a faster sweep speed. J shows the single spike potential (latent period, 2.4 msec) produced by a juxta-fastigial (JF) stimulus. The same voltage scale obtains throughout and the same time scale for A-G and J. In K the reciprocal of each cycle of the rhythmic responses A-D and F, G has been plotted as frequency against as abscissae the time between the stimulus and the end of that cycle. A single curve has been drawn through the points derived from F and G.

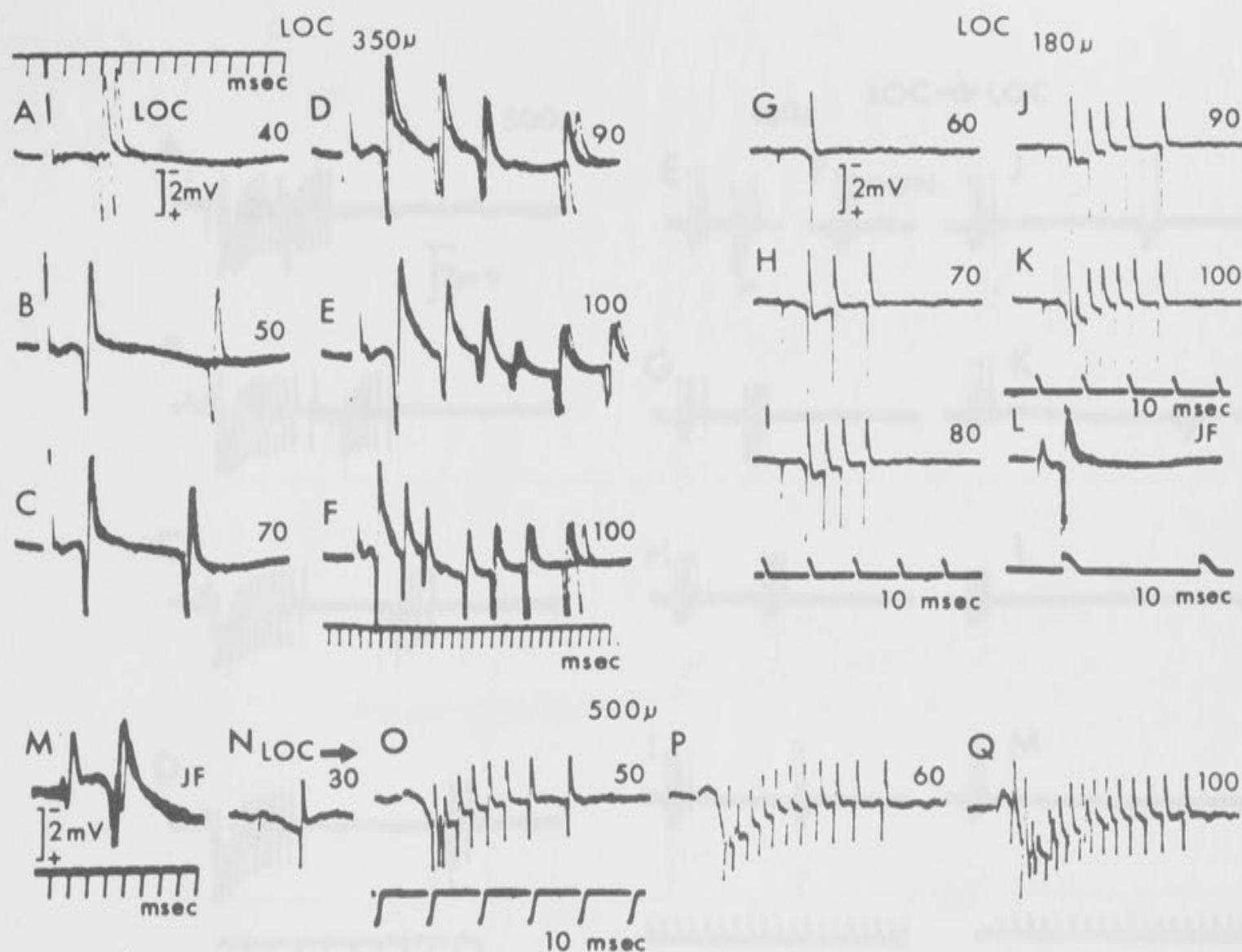


Fig. 44.

Extracellular recording of responses of presumed inhibitory interneurons at depths of 350 μ (A-F), 180 μ (G-L) and 500 μ (M-Q). Just as in Fig. 43 A-G, the responses A-E were evoked by parallel fibre volleys of progressively increasing strength (given in arbitrary units), but from another interneurone. F was at the same strength as E, but at a lower sweep speed. G-K are a similar series of responses to progressively increasing parallel fibre volleys in a more superficial interneurone, L giving the response to juxta-fastigial stimulation (1.9 msec latent period) at a faster sweep speed. N-Q gives also a similar series of responses, but of a deeper interneurone, N showing its response (2.6 msec latent period) to juxtafastigial stimulation at a faster sweep.

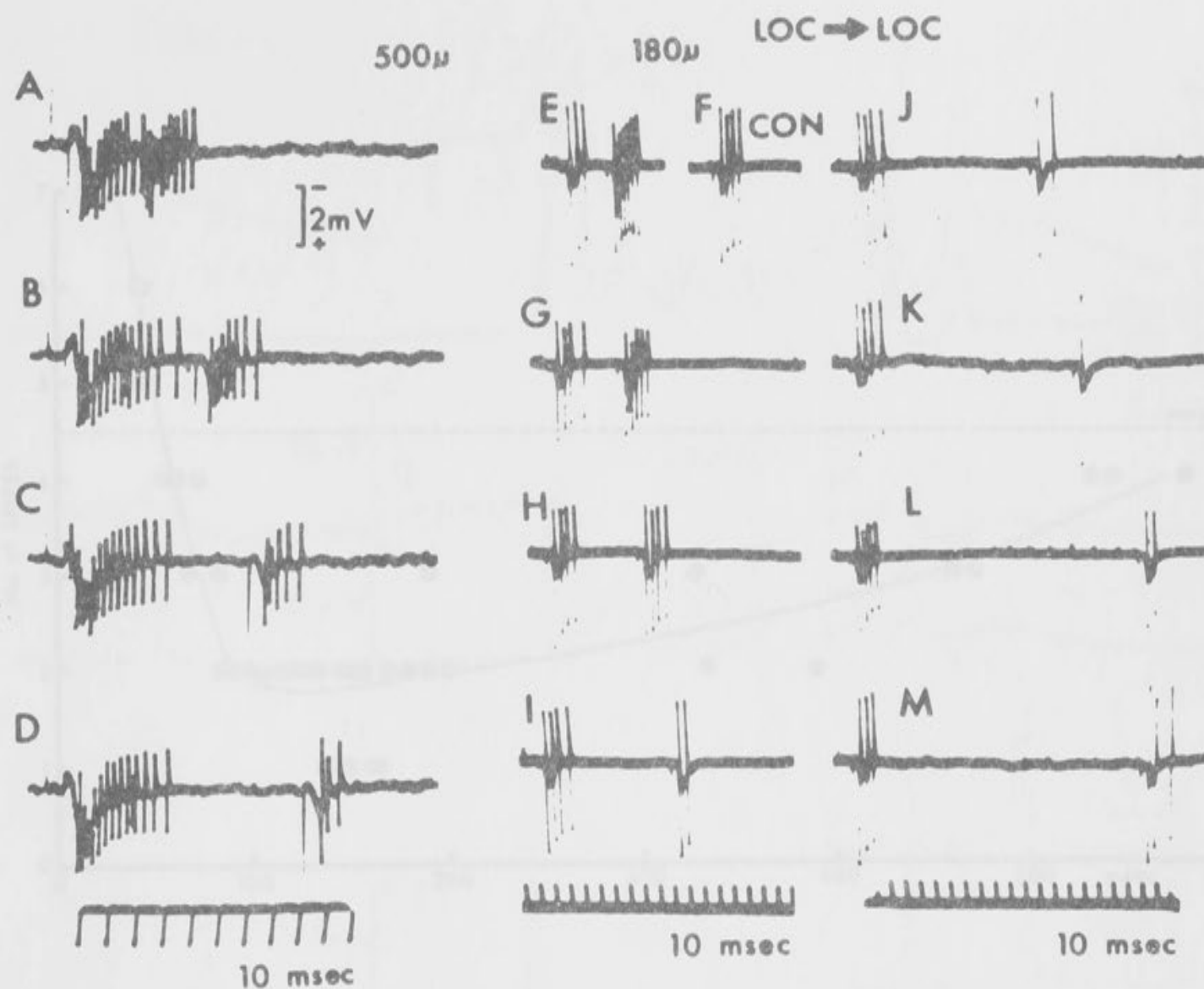


Fig. 45.

Extracellular records of responses evoked in presumed inhibitory interneurons by two parallel fibre volleys. A-D illustrates the potentials from an interneurone recorded at 500_{μ} depth (possibly a Golgi cell). The conditioning and testing stimuli were kept constant and only the stimulus interval varied. The summation of the two volleys is quite apparent.

E-M were recorded in the same interneurone as Fig. 44 G-L at a depth of 180_{μ} . As in A-D the conditioning and testing stimuli were kept constant and the interval between them varied. The control testing response had either 4 spikes as in F or 5 spikes. In I-M there is a reduction of the number of spikes evoked by the second parallel fibre (see text).

Series A-D and E-M had different time scales but the voltage scale is the same for both series.

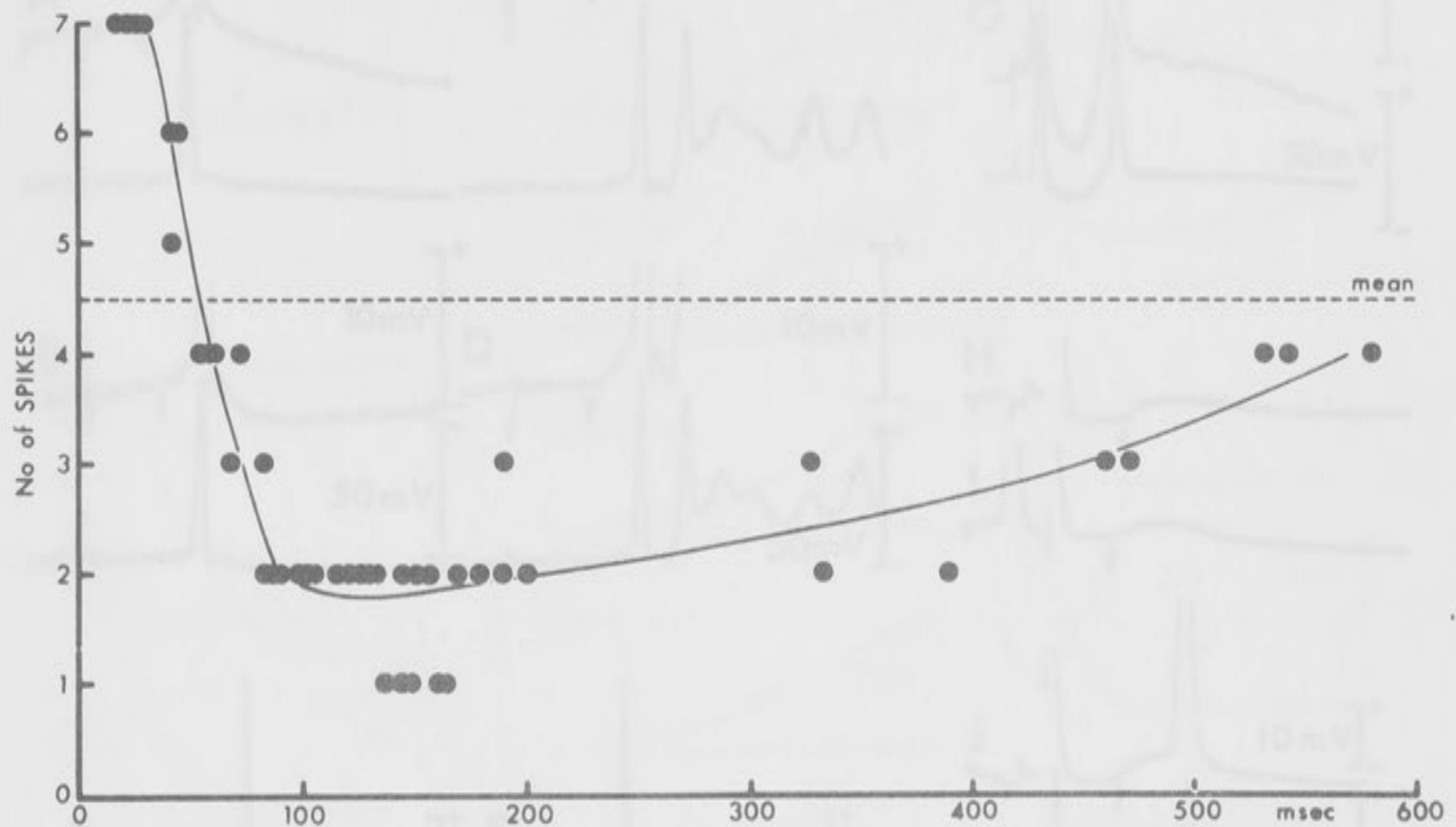


Fig. 46.

Time course of the facilitation and inhibition produced by a parallel fibre volley on a presumed inhibitory interneurone. The series is partly illustrated in Fig. 45 E-M, and the number of spikes produced by the testing parallel fibre volley is plotted against the stimulus interval. There were in all 15 control responses, with 5 spikes in S and 4 spikes in 7 (cf. Fig. 45F).

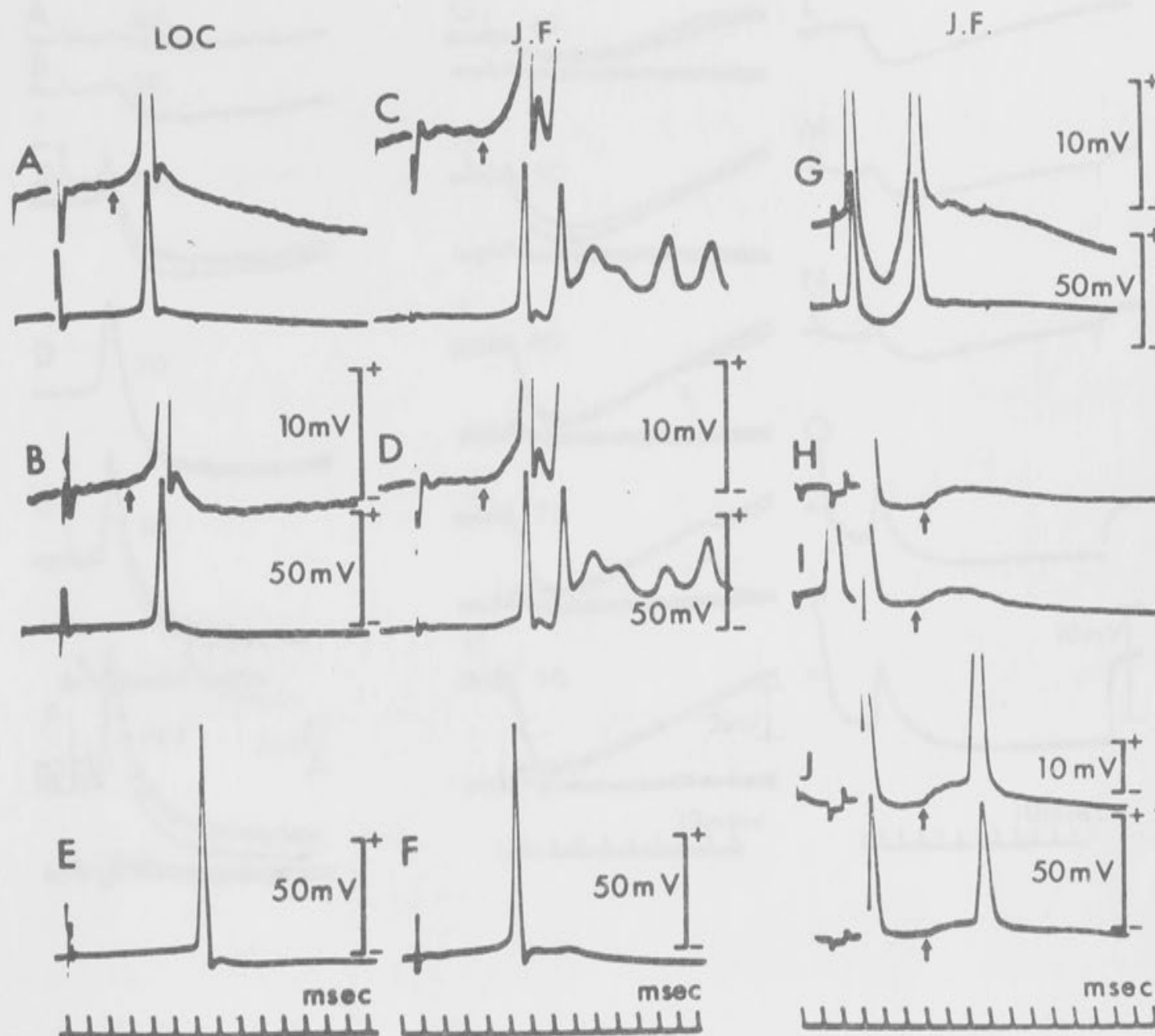


Fig. 47.

Excitatory action evoked by LOC stimulation upon Purkinje cells.

In A and B. intracellularly recorded spike potentials evoked by LOC. stimulations. The upper records are taken at higher amplifications, C and D are similar records evoked by J. F. stimulation, and being followed by typical climbing fibre potentials. Records E and F were evoked by LOC and J. F. stimulation respectively on another Purkinje cell. Note the different firing levels for these two responses.

In G antidromic invasion of a Purkinje cell and the orthodromic activation through the M. G. R. following J. F. stimulation. In H, I and J, graded EPSP produced by a parallel fibre volley following a LOC stimulation. Arrows indicate initiation of parallel fibre EPSPs on to the Purkinje cells. Note time and voltage calibrations.

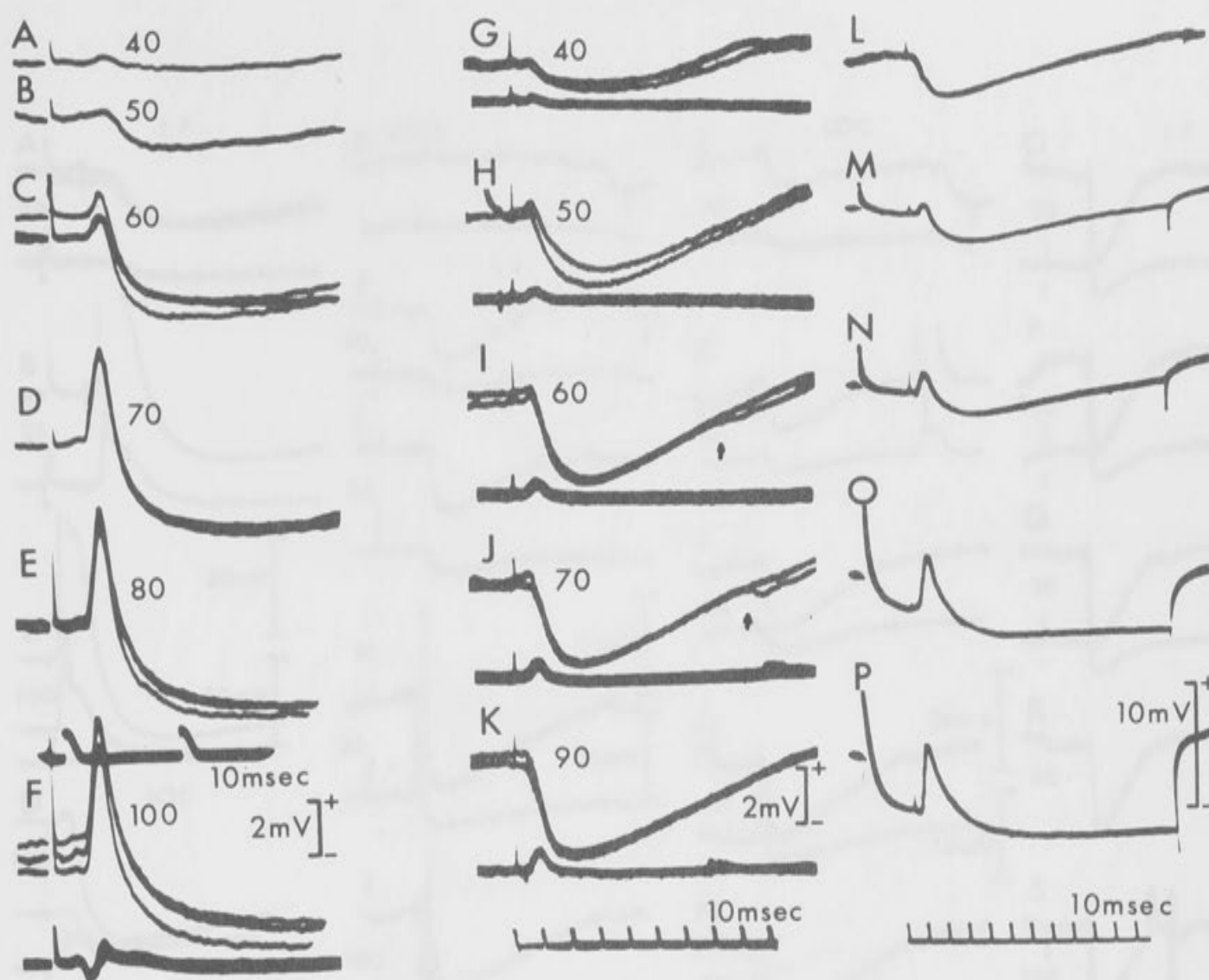


Fig. 48.

Intracellularly recorded synaptic potentials evoked by parallel fibre activation on a Purkinje cell.

Records E to F are possibly recorded from a Purkinje cell dendrite, the EPSP-IPSP sequence are evoked by LOC stimulations of increasing strengths (numerals represent relative values of the stimulus strength).

In F (lower record) the extracellular field potential recorded immediately outside the Purkinje cell illustrated in this series, at a stimulus strength of 100.

In records G-K similar series in another Purkinje cell.

In L-P the action of a hyperpolarizing current applied through the microelectrode on the IPSP illustrated in record R.

The voltage and time calibrations are different for the three series.

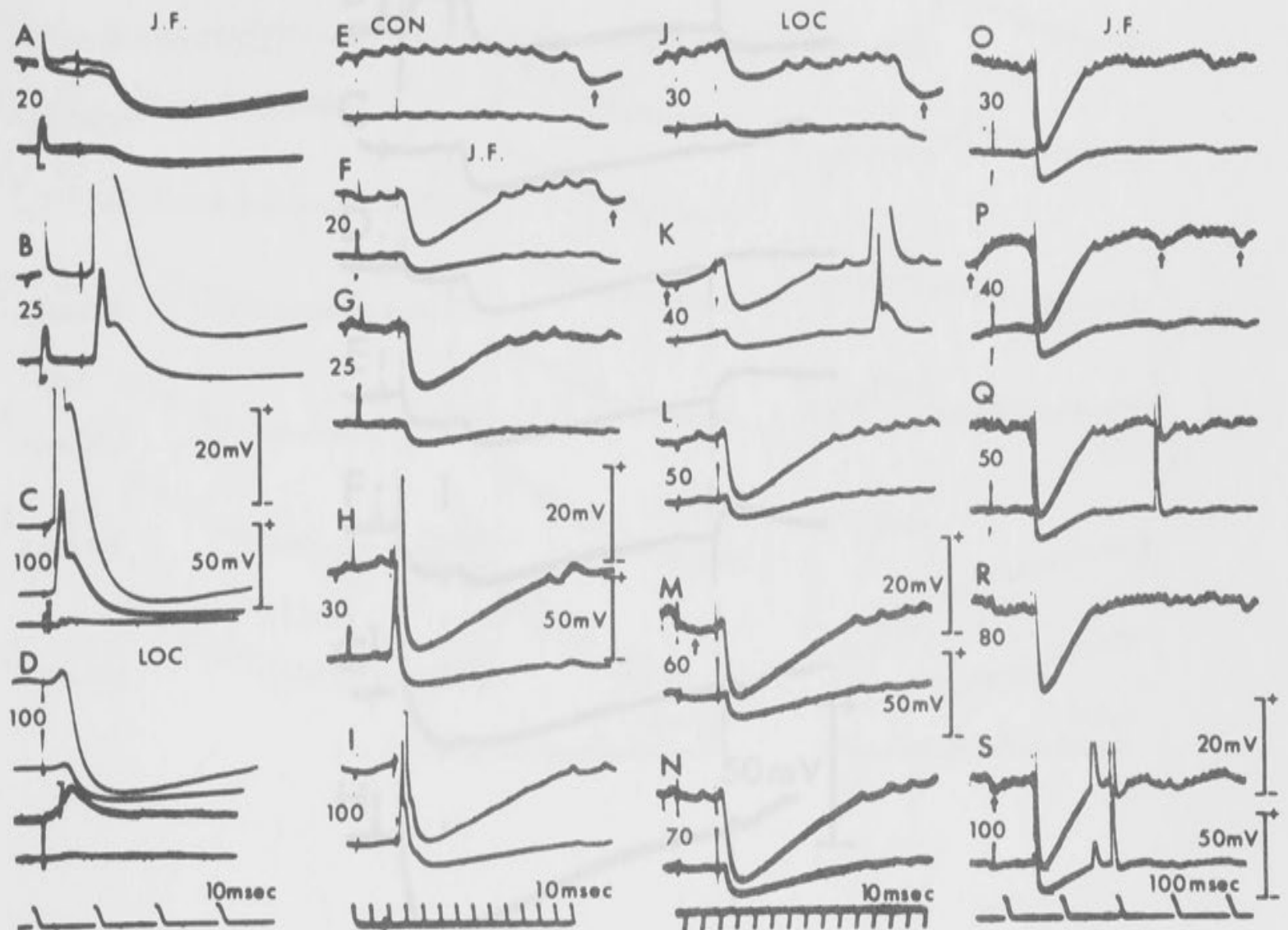


Fig. 49.

Comparison between the synaptic potentials generated by LOC and J. F. stimulation on a Purkinje cell.

In A-C, potentials evoked by J. F. at three different stimulus strengths, recorded at two different amplifications, in C lower record, extracellular field in lower record.

Records F-I, IPSP evoked by J. F. stimulation E is the control, in J-N similar series as F-I but evoked by LOC stimulation. Similar IPSP are illustrated in O-S at slower sweep speed following J. F. stimulation. Note spontaneous IPSP at arrows, and voltage and amplitude calibrations.

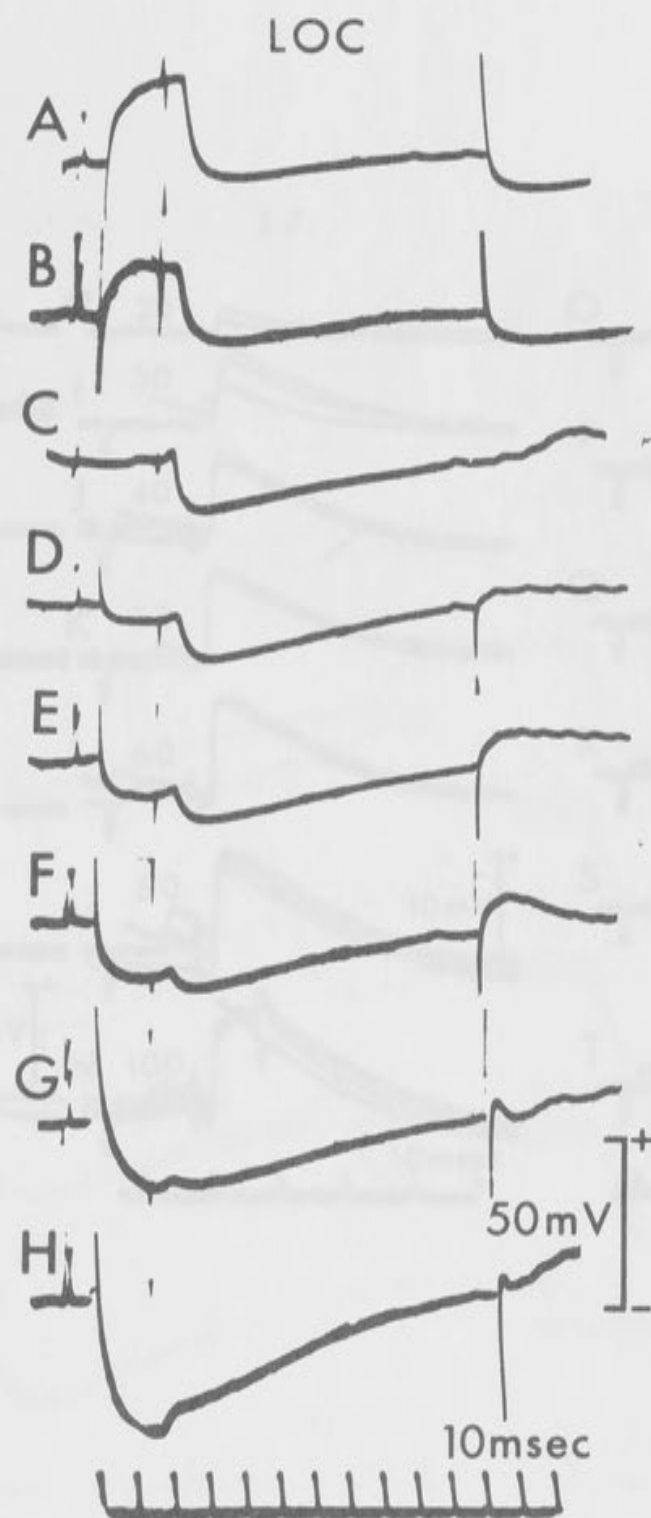


Fig. 50.

Changes produced on an LOC evoked IPSP by the application of currents through the impaling electrode.

Record C is a control IPSP evoked in a Purkinje cell by LOC stimulation. In A and B the cell is depolarized by cathodal current pulse.

In E-H hyperpolarizing currents of increasing magnitudes.

In H there is the initiations of the reversal of the IPSP.

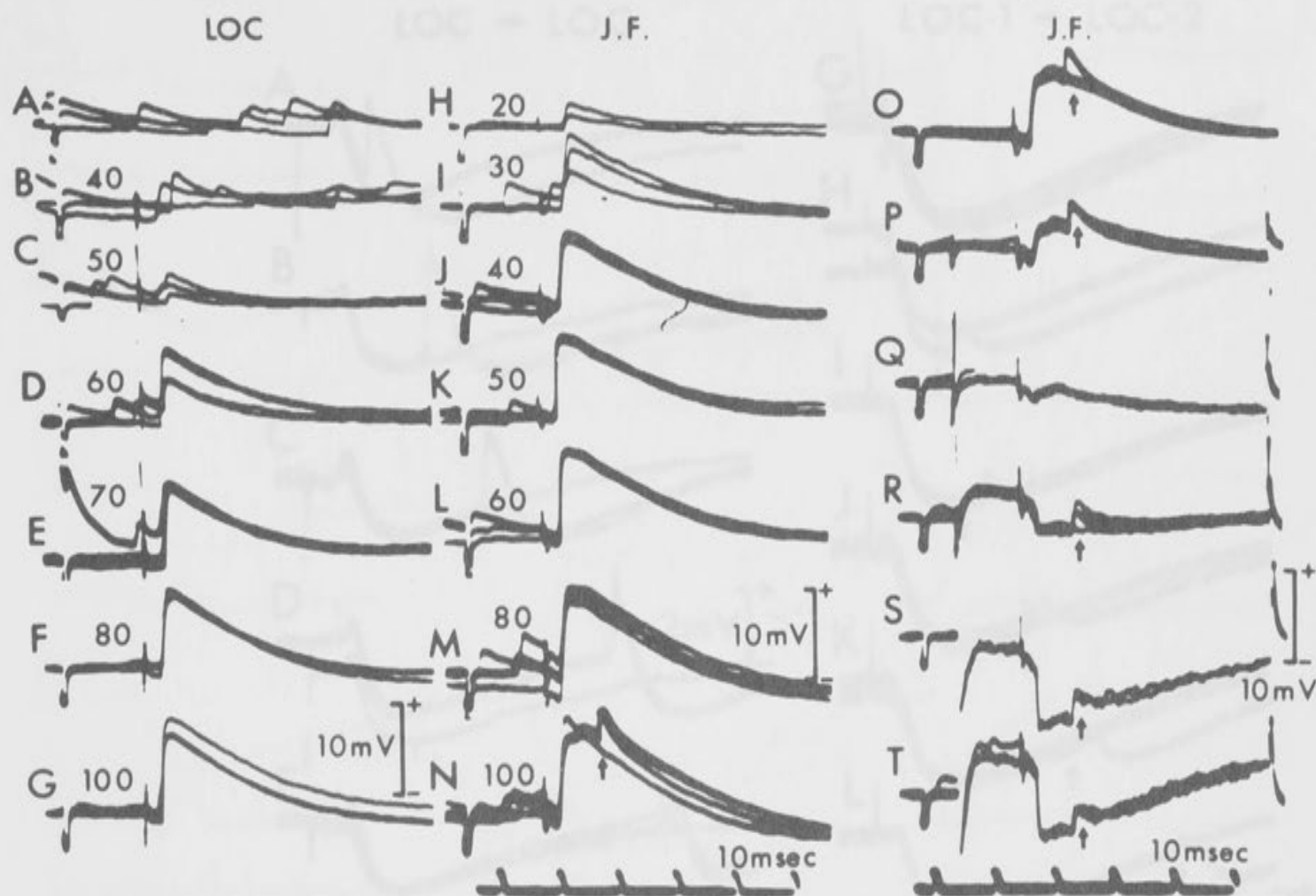


Fig. 51.

Reversal of the LOC and J.F. IPSP by intracellular injections of Cl^- to a Purkinje cell.

Records A-G, depolarizing IPSP evoked by LOC stimulation of increasing strengths (relative values recorded at the left) in a Purkinje cell, after Cl^- injections. In A spontaneous depolarizing IPSP.

Similar J.F. stimulation also evokes reversed IPSP in H-N.

In O-T the depolarized IPSP is re-reversed by the application of depolarizing current through the microelectrode. Note at the arrow, a climbing fibre EPSP which is not reversed in records R-T.

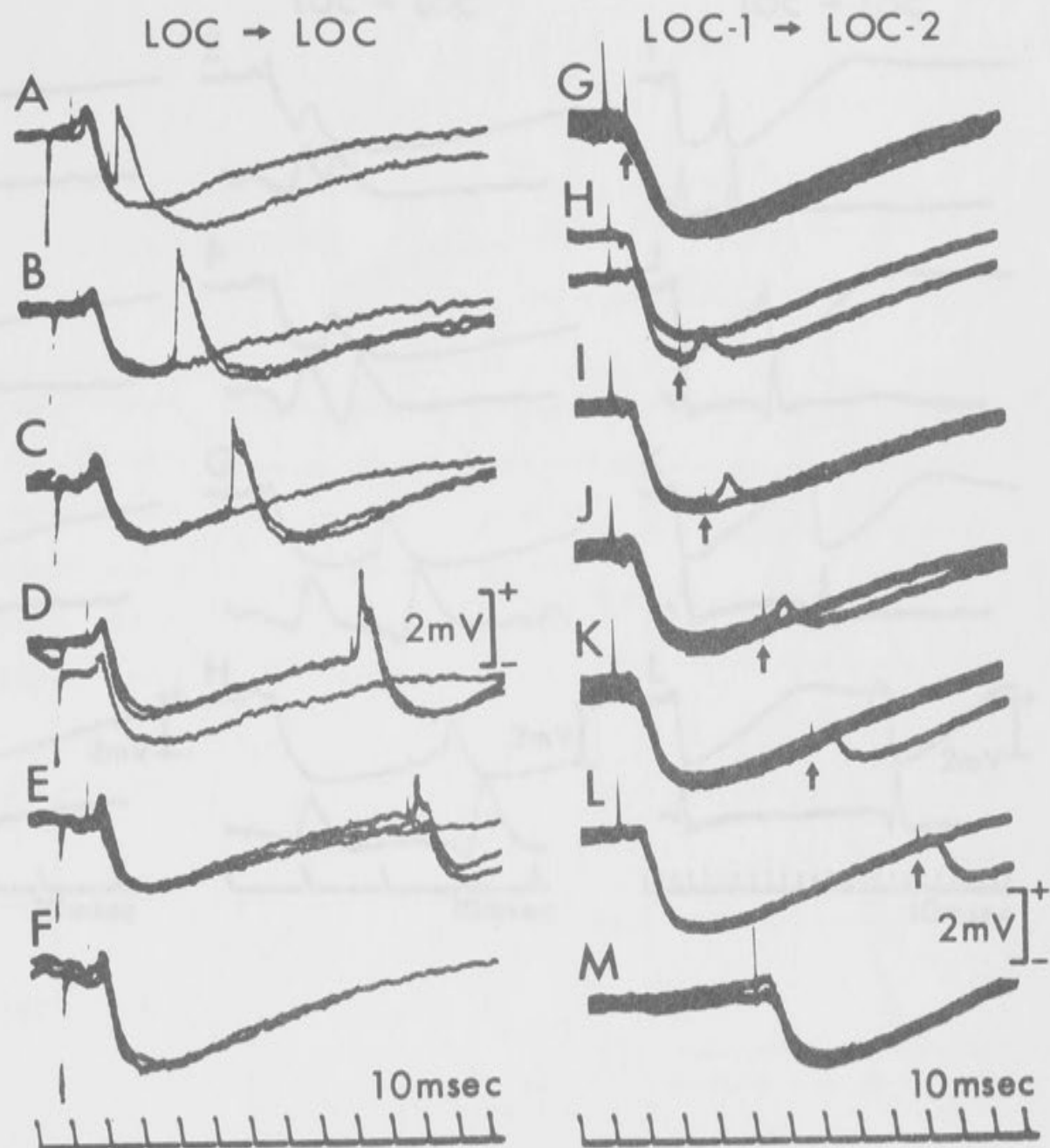


Fig. 52:

Occlusions of a LOC evoked IPSP in a Purkinje cell by a preceding LOC stimulation.

Records A-E shows intracellularly recorded IPSP from Purkinje cell produced by a LOC stimulation and conditioned by a preceding LOC stimulus through the same electrode, at different intervals. F is the control record for the second IPSP.

In G-L, a similar series as in A-E. The conditioning stimulus was applied from a different electrode (LOC_1) than the one applying the test stimulus (LOC_2).

M is the control for LOC_2 stimulation above. Note time and voltage calibration.

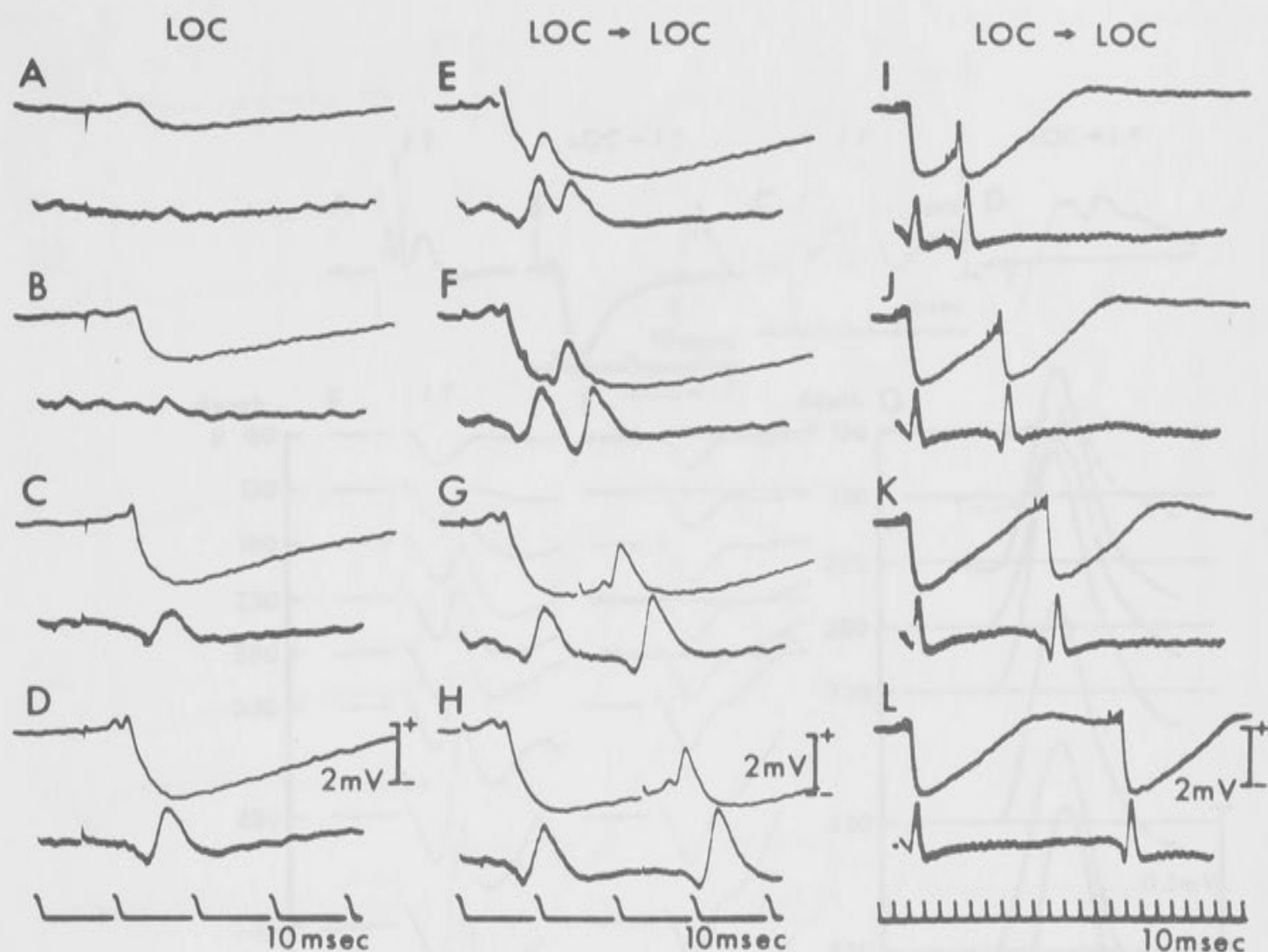


Fig. 53.

IPSP evoked in a Purkinje cell of a totally deafferented cerebellum, by a LOC stimulation.

In A-D IPSPs recorded from a Purkinje cell and evoked by LOC stimulations of increasing strengths. Lower records are the fields potentials recorded from the surface of the cortex by a ball electrode.

Records E-H, IPSP occlusions by a preceding LOC stimulation at different intervals.

In I-L similar records as in E-H, at slower sweep speeds. It will be noted in the lower records traces of record F-L, the time course of the potentiation of the parallel fibre field potential following a LOC stimulation. Note also the delayed depolarization following the IPSP in records I-L.

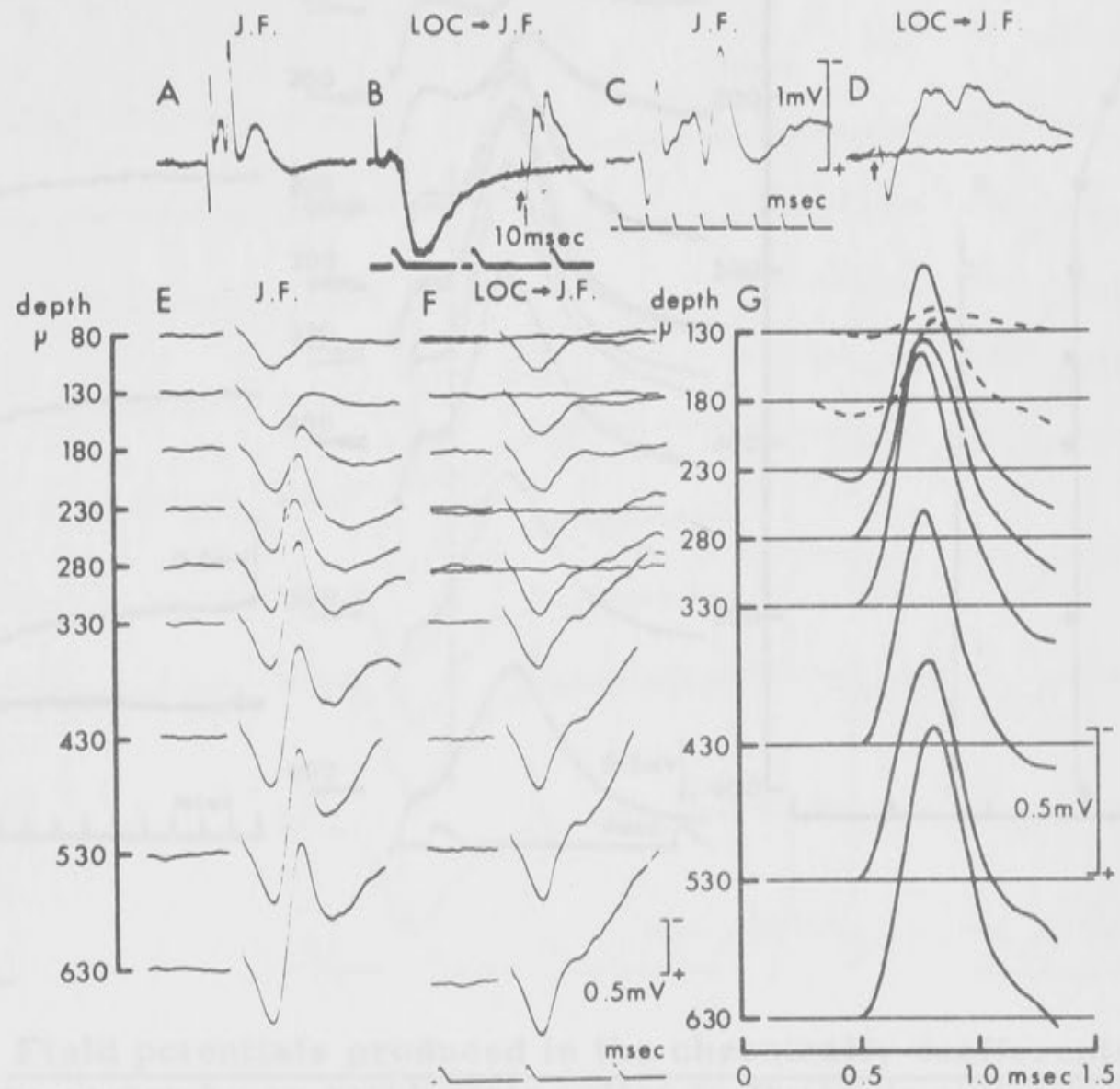


Fig. 55.

Field potentials produced in the normal cerebellar cortex by an antidromic volley in the Purkinje cell axons. A and C show potential evoked at a depth of 330 μ by a J.F. stimulus and recorded at slow and fast speeds, the initial diphasic (positive-negative) spike being due both to impulses in directly stimulated nerve fibres and to the antidromic spike of Purkinje cells. In B the antidromic response (at arrow) was inhibited by a parallel fibre volley 18.3 msec earlier, and in D this inhibited response is shown as the fast sweep superimposed on the base line to the conditioning stimulus alone. In E the antidromic spike potential is shown at a still faster speed and at the indicated depths below the cortical surface, while in F it was inhibited by the preceding parallel fibre volley as in B and D. In G there are plotted at each depth the time courses of the spike potential removed by inhibition, i. e. the difference between the records of E and F. At depths of 130 and 180 μ the subtracted curves are shown as broken lines.

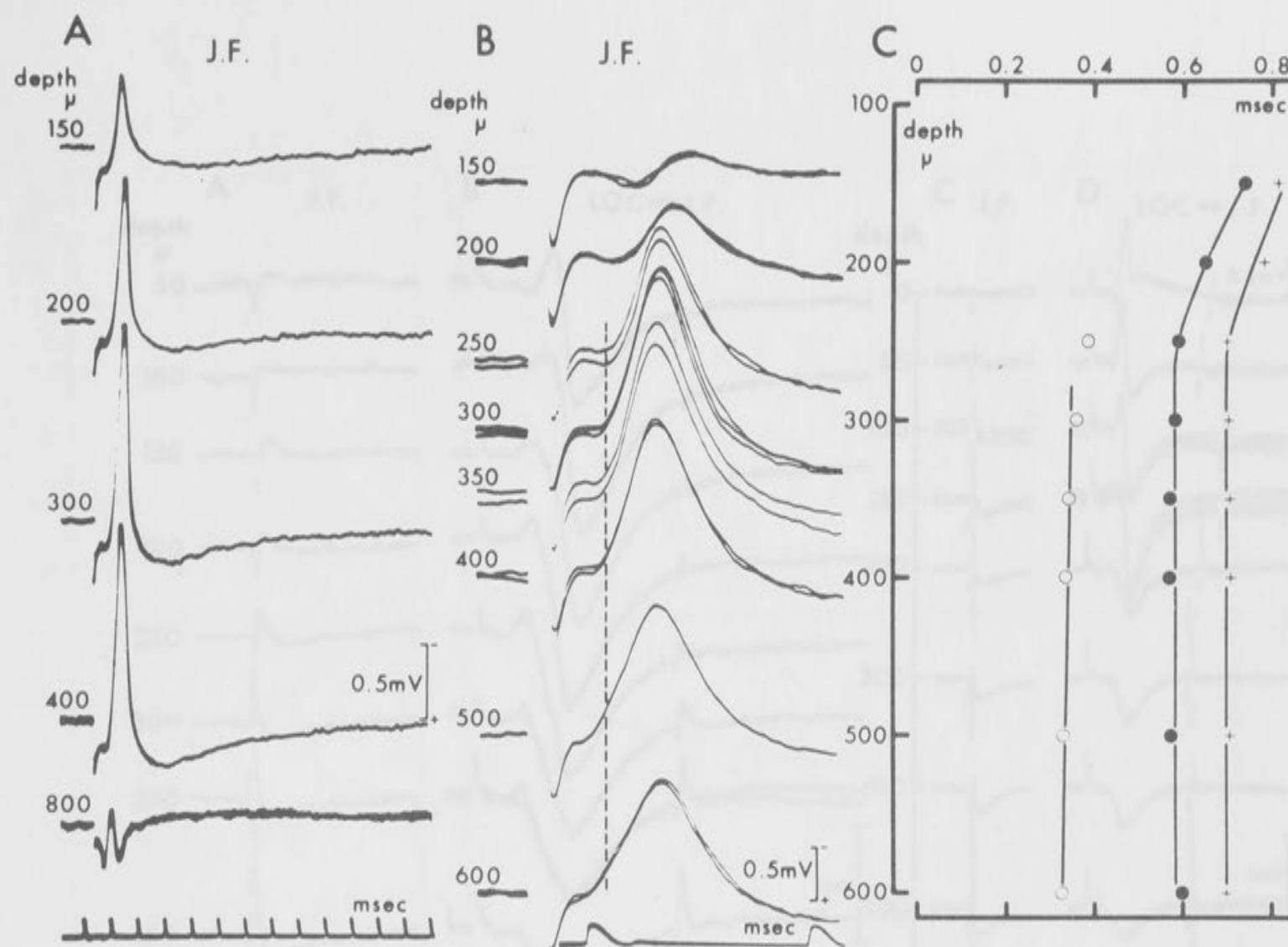


Fig. 56.

Field potentials produced in the chronically deafferented cerebellar cortex by an antidromic volley in Purkinje cell axons.

A. Potentials at the indicated depths below the cortical surface showing the typical positive-negative spike and the slow positive wave at depths of 150 μ to 400 μ .

B. Spike potentials in another experiment at very fast sweep speed in order to allow accurate comparison of the rising phases and summits at the different depths. The vertical line at a latency of 0.35 msec shows the progressively delayed onset of the negative spike at more superficial levels.

C. Measurements from B are plotted to show no significant difference in time to summit (filled circles) from depths of 600 μ to 250 μ , and the progressive increase more superficially. On the other hand (open circles) there was a progressively longer latency from 600 μ to the surface when it was measured to a fixed voltage (a negative deflection of 0.15 mV) on the rising phase. The crosses also show the constant latency of the antidromic spike summit when set up by another J. F. stimulus in this same experiment.

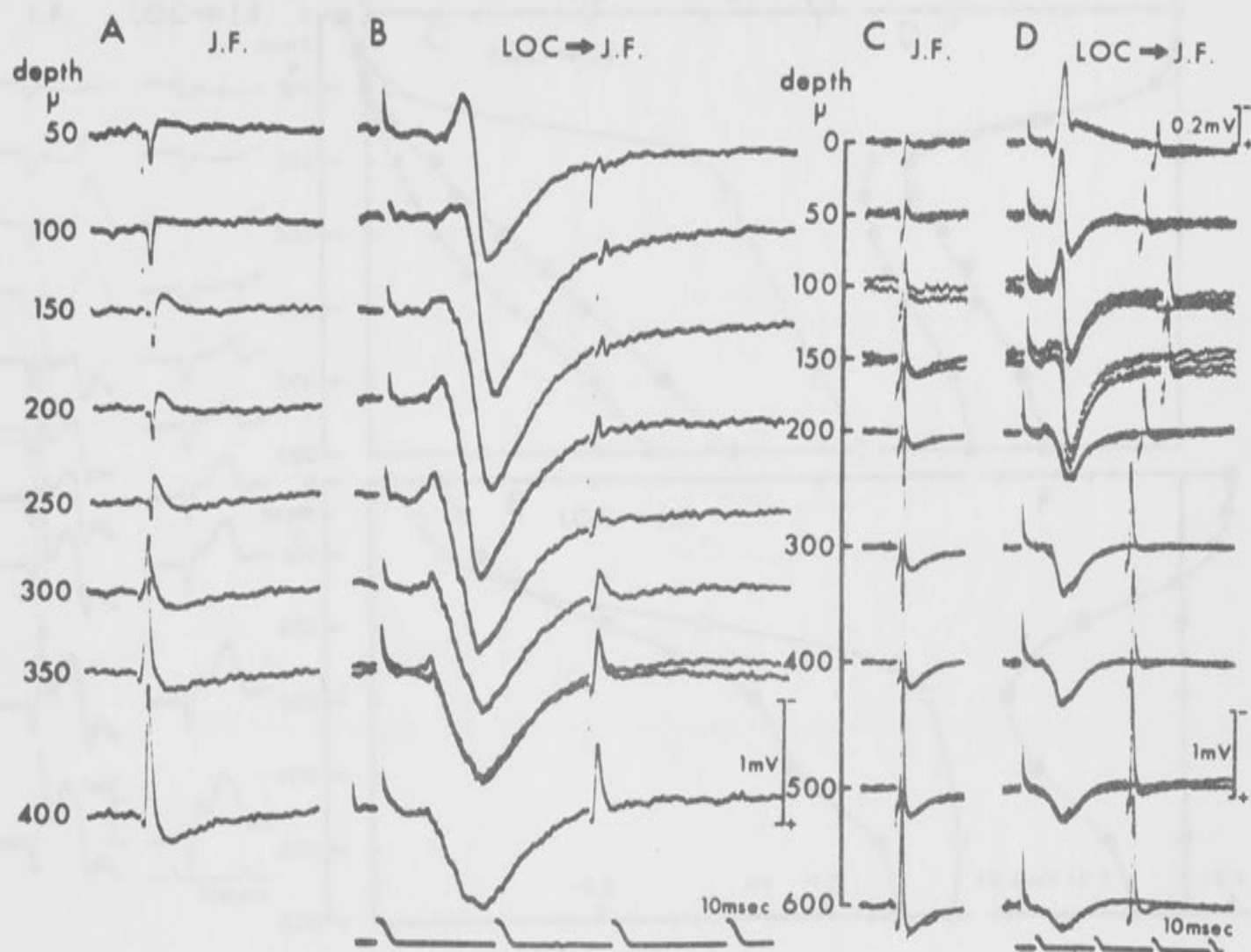


Fig. 57.

Inhibitory and facilitatory influences of a parallel fibre volley on the antidromic spike potential in the cortex of a chronically deafferented cerebellum. A shows potential fields set up by a J.F. stimulus at the indicated depths below the cortical surface. Note inversion at superficial levels as described in text. In B this potential was conditioned by a parallel fibre volley 18 msec earlier, facilitation was signalled by a re-reversal (see text). C and D were obtained in another deafferented cerebellum just as A and B except that the stimulus interval varied from 18 to 24 msec.

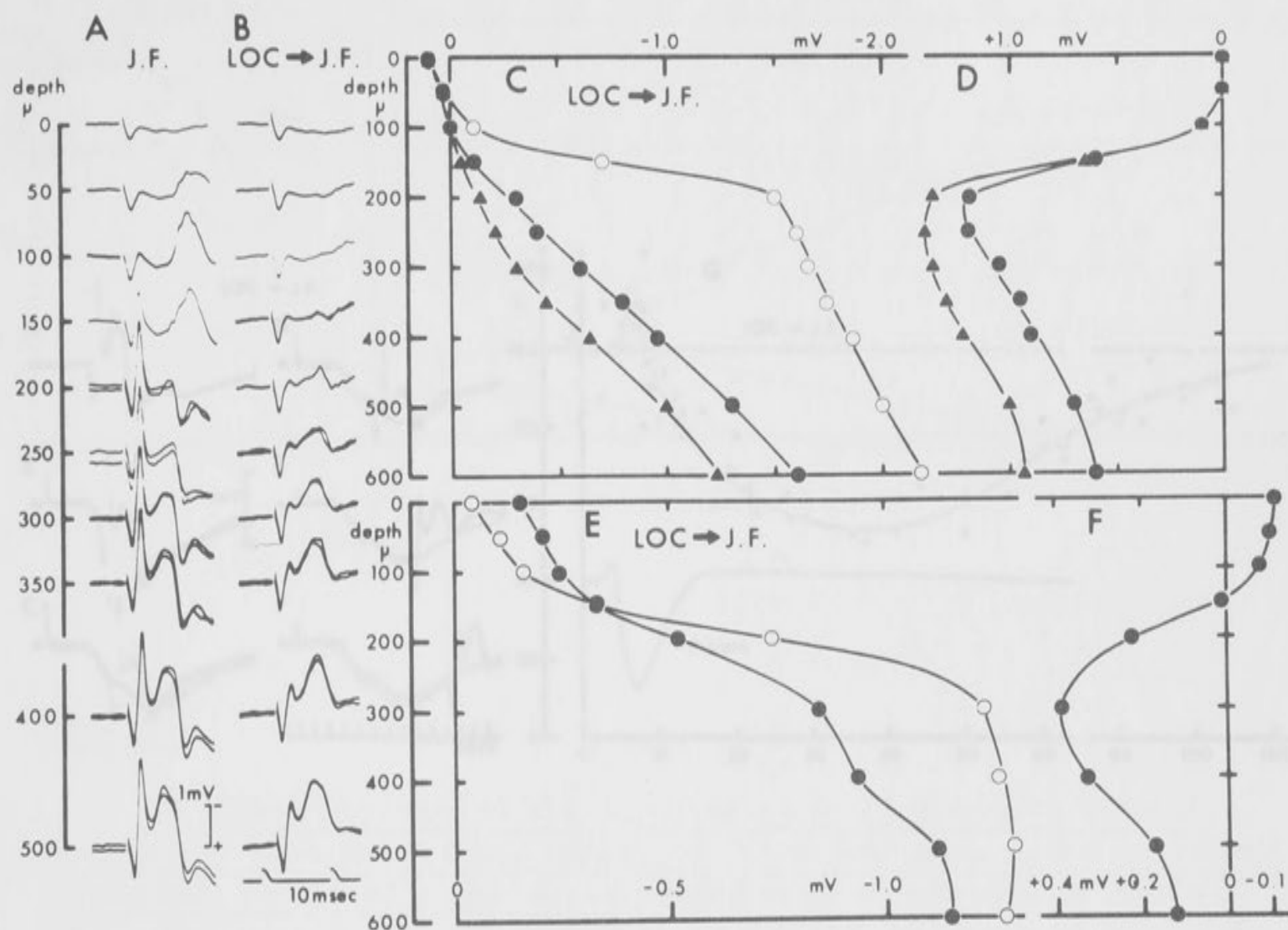


Fig. 58.

Depth profile of the field potential generated by an antidromic volley in Purkinje axons. In A a J.F. stimulus evoked a complex potential wave at the indicated depths below the cartical surface. The initial diphasic (positive-negative) component is the antidromic spike potential of Purkinje cells (cf. Fig. 55) plus the spike potentials in the directly stimulated mossy and climbing fibres. The observations of B were produced concurrently with those of A by the same J.F. stimulus, but were conditioned by a parallel fibre volley 18 msec earlier, just as in Fig. 55B and D. In C the heights of the negative components of the antidromic spike potentials are plotted against the depths for the series of A (open circles) and B (triangles) and also (filled circles) for a series similar to B, but with conditioning by a parallel fibre volley evoked by a weaker stimulus (half strength). In D there are similar depth profile plots for the amount of inhibition by these two sizes of parallel fibre volley, which is expressed as the difference between the control and the inhibited responses. In E there is a similar plot for the depth profile of the control and the inhibited antidromic spike potentials as in C and in F of the amount of inhibition as in D for a similar investigation on a chronically deafferented cerebellum. Specimen records are illustrated in Fig. 57 C and D.

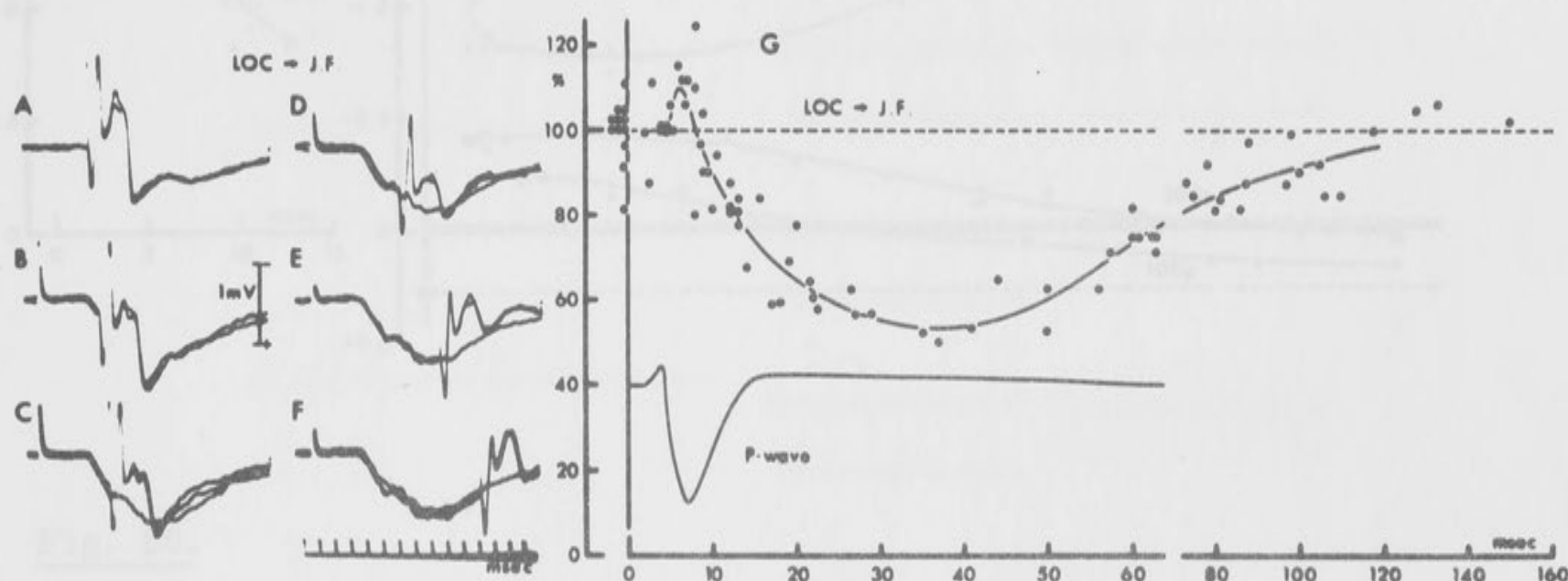


Fig. 59.

Time course of the action exerted by a parallel fibre volley on the antidromic spike potential in the cerebellar cortex.

A shows the typical complex potential produced by a J.F. stimulus in a normal cerebellum and recorded at a depth of 500_{μ} . In B-F this response was conditioned by a parallel fibre volley at various stimulus intervals, there being superimposed in C-F a trace of the response to the conditioning volley alone in order to aid measurements of the size of the negative component of the antidromic spike. The sizes of similar potentials in another experiment are expressed as percentages of the mean control responses and are plotted against the stimulus intervals in G. Note the change in abscissal scaling at the interruption of the base line at 70 msec. In G there is also a tracing showing on the same time scale the P wave that was produced by the conditioning parallel fibre volley as in C to F.

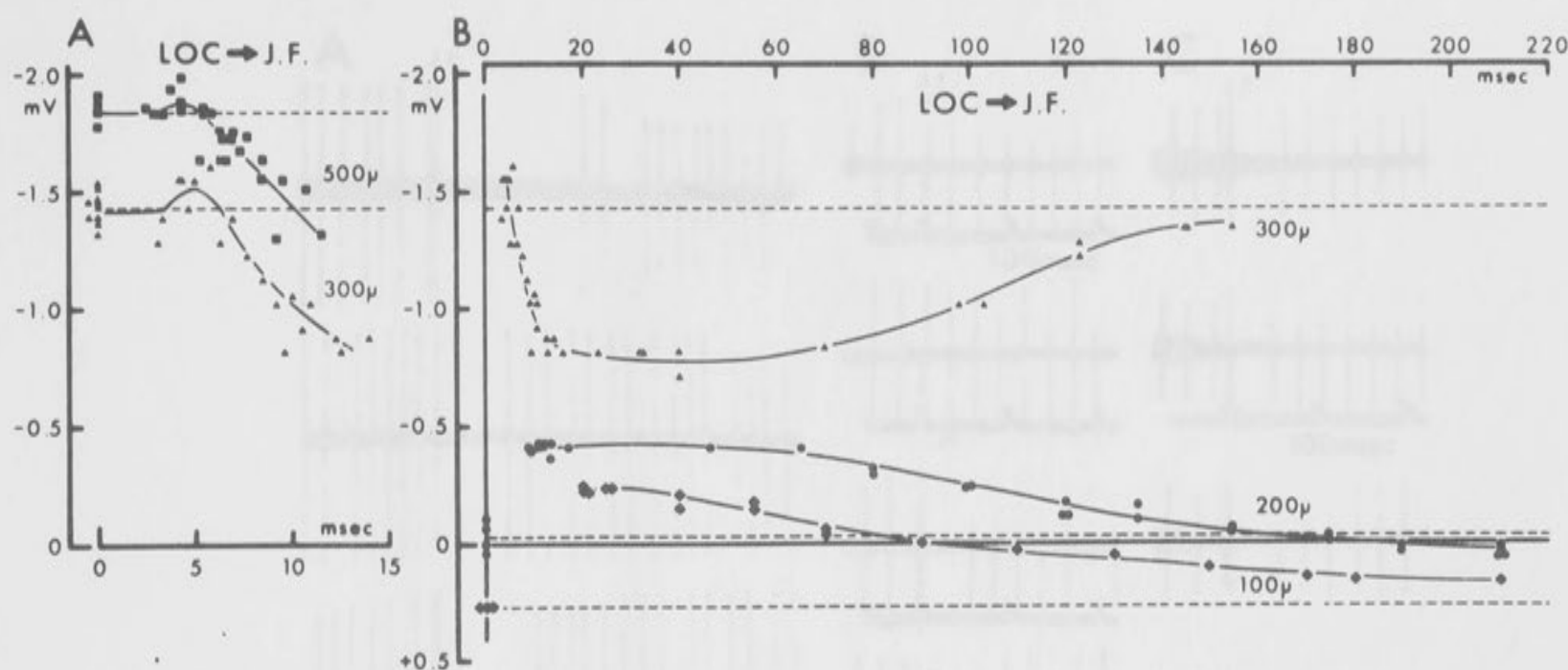


Fig. 60.

Time courses of the facilitatory and inhibitory actions exerted by a parallel fibre volley on the antidromic spike potentials at various levels of a chronically deafferented cerebellar cortex. The antidromic spike potentials were similar to those of Fig. 57A, B in that the dominant negative phase of the antidromic spike potential reversed to a positive spike potential at superficial levels such as 100 μ , the 200 μ depth being transitional, but the experiment was on another chronically deafferented cerebellum. In A the sizes of the conditioned antidromic spike are plotted as in Fig 59 G against stimulus intervals of up to 14 msec for depths of 500 μ (crosses) and 300 μ (filled triangles). In B there is a similar plotting of the conditioned antidromic spike potential for intervals up to 210 msec and for depths of 300 μ (filled triangles), 200 μ (filled circles) and 100 μ (open circles). Note that the mean control potentials in B were about zero at 200 μ and positive at 100 μ . Note also that at 100 μ the facilitatory influence converts the positive spike to a negative spike, from below to above the zero line, as is illustrated at the superficial levels of Fig. 57 B.

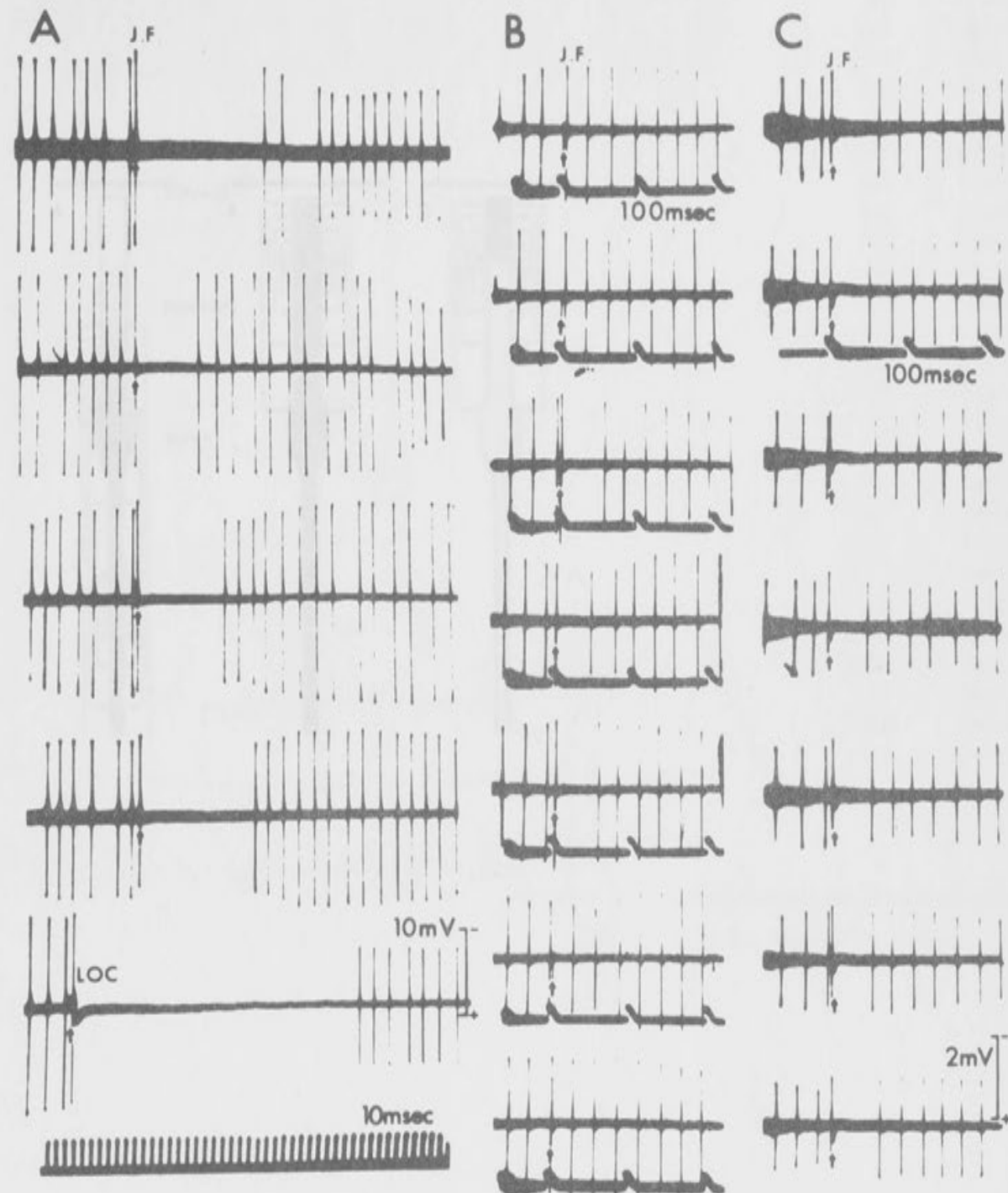


Fig. 61.

Inhibitory action on the spontaneously rhythmic discharges of Purkinje cells in the chronically deafferented cerebellum. In the four upper records of A a single J.F. stimulus was applied at the arrows and evoked an antidromic response of the Purkinje cell followed by a silence of variable duration. In the lowest record a much longer silence was produced by a single parallel fibre volley. The variations in the spike lengths are due to slight movements caused by respiration. B and C illustrate the weaker inhibition that a J.F. stimulus exerts on a rhythmic Purkinje cell in another chronically deafferented cerebellum. In B the stimulus was just at threshold and in this consecutive series of traces it excited the Purkinje cell antidromically only in the middle three. In C the J.F. stimulus was 2.5 times the threshold strength and on every occasion an antidromic spike was set up followed by a fairly long silence.

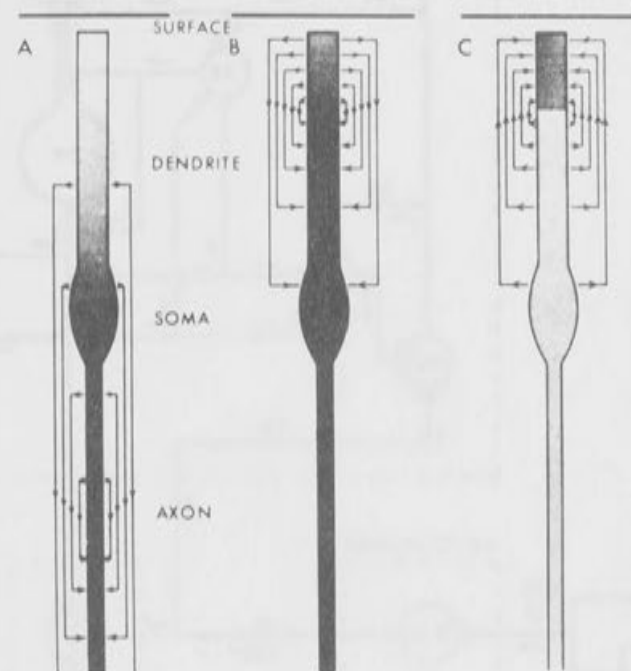


Fig. 62.

Diagrams to show field potentials generated by the antidromic propagation of an impulse into a Purkinje cell.

The grey shading indicates depolarization, the darker the grey the more intense the depolarization. The zones occupied by the impulse are shown black. A single Purkinje cell is shown in A, B and C, but the lines of extracellular current flow are drawn confined to the immediate surround, as they would be in the situation where all Purkinje cells in an area are being simultaneously invaded. In A the antidromic impulse is propagating up the axon and there is a graded electrotonic depolarization of the soma and dendrites. In B the impulse has invaded the soma and dendrites to the maximum extent, there being a terminal dendritic zone not invaded, but merely depolarized by the electrotonic current. In C the axon, soma and the invaded part of the dendrites have almost completely recovered from the impulse, with the consequence that those regions are less depolarized than the uninvaded dendritic zone; hence the reversed current flow.

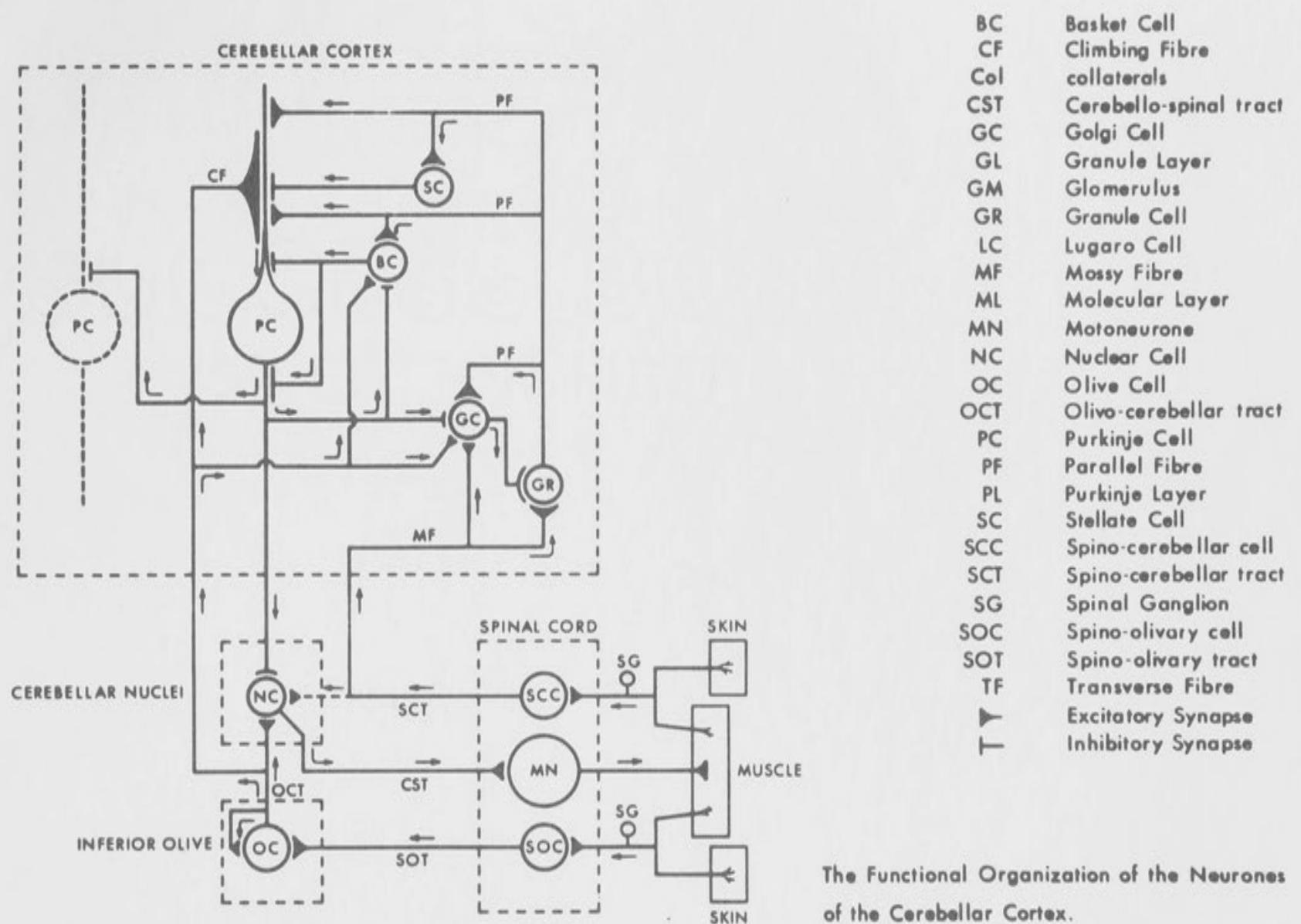


Fig. 63.

The diagram illustrates the over all conclusion reached in the present study concerning the synaptic organization of the neurones in the cerebellar cortex. Some of the relations of this cortex with other regions of the central nervous system are also illustrated. Note the different signs used for excitatory and inhibitory synaptic endings.